PNAS Plus Significance Statements

Stress and telomere shortening among central Indian conservation refugees

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Recent research links life stress to premature telomere shortening and human aging. However, this association has only been demonstrated in Western contexts, where stress is typically lower and life expectancies longer. Using innovative approaches, we show significant associations between stress and telomere shortening in a non-Western setting among a highly stressed population with lower life expectancies: poor indigenous people—the Sahariya—who were displaced between 1998 and 2002 from their central Indian wildlife sanctuary homes. Our research (pp. E928–E936) strengthens the case for stress-induced telomere shortening as a pancultural biomarker of compromised health and aging.

Multimodular biosensors reveal a novel platform for activation of G proteins by growth factor receptors

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Long-held tenets in the field of signal transduction are that G proteins are activated exclusively by G protein-coupled receptors and that growth factor receptor tyrosine kinases (RTKs) do not have the wherewithal to do the same. In this work (pp. E937–E946) we created fluorescent biosensors derived from the multimodular signal transducer Gα-interacting vesicle-associated protein (GIV), an unusual protein that binds RTKs and activates G proteins, and used them in FRET and bimolecular fluorescent complementation assays to visualize RTK–GIV–G protein signaling complexes directly in single living cells. These studies not only provide evidence that GIV serves as a platform for transactivation of G proteins by growth factor RTKs but also illuminate the spatial and temporal dynamics of such noncanonical G protein signaling.

Integrity of the yeast mitochondrial genome, but not its distribution and inheritance, relies on mitochondrial fission and fusion

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Mitochondrial DNA (mtDNA) encodes essential subunits of respiratory complexes, which are responsible for the generation of ATP through oxidative phosphorylation in mitochondria. Copies of mtDNA are distributed throughout the mitochondrial network and are faithfully inherited during the cell cycle. We have developed a novel tool in *Saccharomyces cerevisiae* that allows us to watch mtDNA dynamics in living cells and to characterize its distribution and inheritance. We show that, surprisingly, mitochondrial fusion and fission are dispensable for both processes. The absence of fusion and fission events, however, leads to the accumulation of rearranged and dysfunctional mitochondrial genomes. These results (pp. E947–E956) reveal crucial roles of mitochondrial fusion and fission in maintaining the quality and integrity of the mitochondrial genome.

Dendritic cell SIRT1–HIF1α axis programs the differentiation of $CD4^+$ T cells through IL-12 and TGF-β1

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Naive $CD4^+$ T cells differentiate into diverse effector and regulatory subsets to orchestrate immunity and tolerance. Whereas the mechanism of T-cell intrinsic signals has been extensively studied, how T-cell lineage differentiation is controlled by innate immune signals remains unknown. Here (pp. E957–E965) we used loss-of-function mouse systems, combined with other complementary approaches and models, to define the role of dendritic cell (DC) sirtuin 1 (SIRT1) as a key regulator in orchestrating the orientation of T-cell differentiation via HIF1α signaling in a mammalian target of rapamycin–independent manner. DC-expressed SIRT1, a type III histone deacetylase, programmed reciprocal T helper 1 (T_H1) and regulatory T-cell (T_{reg}) differentiation by modulating IL-12–STAT4 and TGF-β1–SMAD3 axes and cytokine receptor expressions at the DC–T-cell interface.

Therapeutic antitumor immunity by checkpoint blockade is enhanced by ibrutinib, an inhibitor of both BTK and ITK

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Antibodies that block the negative signals between PD1-Ligand on tumor cells and PD-1 on T cells are effective therapies against several types of cancer. Ibrutinib, a covalent inhibitor of BTK is an approved therapy for B-cell leukemia and lymphoma. But ibrutinib also inactivates ITK, an enzyme required for certain subsets of T lymphocytes (Th2 T cells). We found that the combination of anti–PD-L1 antibodies and ibrutinib led to impressive therapeutic effects not only in animal models of lymphoma but, surprisingly, also in models of breast cancer and colon cancer. Based on these preclinical results (pp. E966–E972), we suggest that the combination of PD-1/PD-L1 blockade and ibrutinib be tested broadly in patients with lymphoma and also in other hematologic malignancies and solid tumors.

iASPP, a previously unidentified regulator of desmosomes, prevents arrhythmogenic right ventricular cardiomyopathy (ARVC)-induced sudden death

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a disease that is selective to the right side of the heart and results in heart failure and sudden death. Genetic defects in desmosome components account for approximately 50% of human ARVC cases; in the other 50% of patients, however, the causes remain unknown. We show (pp. E973–E981) that inhibitor of apoptosis-stimulating protein of p53 (iASPP) is an important regulator of desmosomes. It interacts with desmoplakin and desmin in cardiomyocytes and regulates desmosome integrity and intermediate filaments. iASPP-deficient mice display pathological features of ARVC and die of sudden death. In human ARVC patients, cardiomyocytes exhibit reduced levels of iASPP at the cell junctions, suggesting that iASPP may be critical in ARVC pathogenesis.

Antisense long noncoding RNAs regulate *var* gene activation in the malaria parasite *Plasmodium falciparum*

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How cells specifically express only a single gene among numerous equivalent copies within their genomes is one of the unsolved mysteries in the field of eukaryotic gene expression. The molecular mechanisms that underlie mutually exclusive gene expression are the key for understanding the virulence of *Plasmodium falciparum*, the parasite responsible for the deadliest form of human malaria. *P. falciparum* expresses its primary virulence determinants in a mutually exclusive manner and evades human immune attack through switches in expression between different variants of a large gene family named *var*. We found that *var*-specific antisense long noncoding RNA molecules incorporate into chromatin and determine how parasites select a single gene for expression while the rest of the family is maintained silenced (pp. E982–E991).

Balance of cellular and humoral immunity determines the level of protection by HIV vaccines in rhesus macaque models of HIV infection

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Our candidate HIV vaccine, a single-chain gp120-CD4 chimera, elicits protection against acquisition of simian-human immunodeficiency virus (SHIV)/simian immunodeficiency virus (SIV) in rhesus macaques (pp. E992–E999). Antibody-dependent cellular cytotoxicity was an inverse correlate of infection risk. However, it is attenuated when antigen-specific T-cell responses exceed a threshold, presumably due to the generation of CD4+ CCR5+ T cells, the preferred cellular targets of SHIV/SIV. Multiple studies strongly suggest that HIV/SIV-specific T-cell responses are a double-edged sword. On one hand, they are required for T-cell help in the protective antibody response. On the other hand, they appear to mitigate protection by creating new targets for viral replication. Determining the balance between protective antibody responses and attenuating T-cell responses is a key challenge confronting HIV vaccine development.

Control of a hair bundle's mechanosensory function by its mechanical load

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Hair bundles are the sensory antennae that detect different types of mechanical signals in diverse sensory systems of vertebrates. Here (pp. E1000–E1009) we design and use a mechanical-load clamp to show that the mechanical properties of hair bundles and their accessory structures dictate their sensory behaviors. By demonstrating how the same organelle can be used to detect a wide gamut of signals, this study reveals both the versatility and essential similarity of hair bundles across receptor organs. These observations reveal a general principle that may be used by both biological and artificial systems: by adjustment of only a few key parameters, a nonlinear system can be controlled to serve many different functions.

Major diversification of voltage-gated K^+ channels occurred in ancestral parahoxozoans

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We examined the origin and evolution of two major families of voltage-gated K^+ channels, Shaker and KCNQ, which regulate action potential repolarization, patterning, and threshold. Shaker family channels evolved in a basal metazoan ancestor of ctenophores and parahoxozoans (including cnidarians and bilaterians), but functional diversification of the Shaker family and the emergence of the KCNQ family occurred specifically within the parahoxozoan lineage. Our results (pp. E1010–E1019) suggest that many major innovations in the regulation of cellular excitability by voltage-gated K^+ channels are unique to parahoxozoans and that these innovations occurred before the divergence of cnidarians and bilaterians. Ctenophores and sponges separated prior to this burst of innovation and thus either lack major mechanisms for action potential regulation or evolved such mechanisms independently.

Essential functions of primate frontopolar cortex in cognition

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The frontopolar cortex (FPC) is a large region occupying the anterior portion of the brain's frontal lobe, and has been suggested to play a role in complex, higher order behavior. However, the specific contributions of this area toward this type of behavior are still unclear. Using an animal model, we show that localized lesions to the FPC of nonhuman primates selectively impair the ability of the animal to learn rapidly about novel objects and rules, although sparing its ability to draw upon previously established knowledge about objects and rules. These findings (pp. E1020–E1027) suggest that the FPC makes a crucial contribution to the exploration and rapid acquisition of novel behavioral options, which is, in turn, an essential aspect of complex, higher order behavior.

EF-hand protein Ca^{2+} buffers regulate Ca^{2+} influx and exocytosis in sensory hair cells

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Ca^{2+} ions serve as a key cellular signal and are tightly controlled. One mechanism to limit free Ca^{2+} ions is buffering by Ca^{2+}-binding proteins, which are strongly expressed in sensory hair cells of the ear. Here (pp. E1028–E1037) we studied how genetic disruption of the Ca^{2+}-binding proteins parvalbumin-α, calbindin-D28k, and calretinin affects exocytosis and sound encoding at the synapses of mouse inner hair cells (IHCs) and spiral ganglion neurons (SGNs). Mutant IHCs showed increased exocytosis, but the sound-evoked spiking activity in SGNs was unaltered. Together with mathematical modeling, this finding indicates that a large fraction of exocytosis in mutant IHCs occurred outside synapses. We conclude that Ca^{2+}-binding proteins shape presynaptic Ca^{2+} signals to restrict exocytosis to active zones, thus enabling metabolically efficient sound encoding.

Mechanistic links between cellular trade-offs, gene expression, and growth

Andrea Y. Weiße, Diego A. Oyarzún, Vincent Danos, and Peter S. Swain

Cells have finite resources. Committing resources to one task therefore reduces the amount of resources available to others. These trade-offs are often overlooked but potentially modify all cellular processes. Building a mathematical cell model that respects such trade-offs and describes the mechanisms of protein synthesis and how cells extract resources from their environment, we quantitatively recover the typical behavior of an individual growing cell and of a population of cells (pp. E1038–E1047). As trade-offs are experienced by all cells and because growth largely determines cellular fitness, a predictive understanding of how biochemical processes affect others and affect growth is important for diverse applications, such as the use of microbes for biotechnology, the inhibition of antibiotic resistance, and the growth of cancers.