PNAS Plus Significance Statements

Tracking sustainable development with a national barometer for South Africa using a downscaled "safe and just space" framework

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We have downscaled planetary boundaries and applied the "safe and just space for humanity" framework at the national scale, for the first time, creating a "barometer" for inclusive sustainable development for South Africa. The barometer presents the state and trajectory of a broad but manageable set of indicators for environmental and social priorities, and highlights the country's proximity to environmental boundaries and the distance from eradication of social deprivation. This creates a monitoring and communication tool for national government for thinking in an integrated manner about environmental and social-development issues. Our case study (pp. E4399–E4408) provides insight into the challenges and complexities of developing indicators and targets for the proposed global Sustainable Development Goals that that are globally, regionally, and nationally relevant.

Multivariate biophysical markers predictive of mesenchymal stromal cell multipotency

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We identify a set of unique biophysical markers of multipotent mesenchymal stromal cell populations. Multivariate biophysical analysis of cells from 10 adult and fetal bone marrow donors shows that distinct subpopulations exist within supposed mesenchymal stem cell populations that are otherwise indistinguishable by accepted stem cell marker surface antigens. We find (pp. E4409–E4418) that although no single biophysical parameter is wholly predictive of stem cell multipotency, three of these together—cell diameter, cell mechanical stiffness, and nuclear membrane fluctuations—distinguish multipotent stem cell from osteochondral progenitor subpopulations. Together, these results (along with the corresponding statistical correlations) show that a minimal set of biophysical markers can be used to identify, isolate, and predict the function of stem and progenitor cells within mixed cell populations.

Conformational coupling between the active site and residues within the K^C-channel of the *Vibrio cholerae* cbb_3-type (C-family) oxygen reductase

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Many pathogenic bacteria require an aerobic respiratory chain that functions at very low oxygen concentrations. This is accomplished by using a variant of cytochrome *c* oxidase known as cytochrome cbb_3. Although related to the human oxidase, cbb_3 has distinct differences that provide opportunities for developing selective drugs. We used (pp. E4419–E4428) site-directed mutagenesis guided and complemented by molecular dynamics simulations to characterize the essential proton channel within the cbb_3 from *Vibrio cholerae*. Several critical residues are identified, and evidence is presented to show that perturbations near the entrance of the proton channel can have dramatic effects at the active site of the enzyme, more than 25 Å away. This is among the features that distinguish the pathogen respiratory enzyme from the human enzyme.

Role of Erbin in ErbB2-dependent breast tumor growth

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ErbB2 (v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2) is overexpressed in around 25% of breast cancers. The present study (pp. E4429–E4438) reveals that Erbin, an ErbB2-interacting protein that was thought to act as an antitumor factor, facilitates ErbB2-dependent proliferation of breast cancer cells and tumorigenesis in *MMTV-neu* transgenic mice. Disruption of the interaction decreases ErbB2-dependent proliferation, and deletion of the PDZ domain in Erbin hinders ErbB2-dependent tumor development in *MMTV-neu* mice. Erbin forms a complex with ErbB2, promotes its interaction with the chaperon protein HSP90, and thus prevents its degradation. ErbB2 and Erbin expression correlates in human breast tumor tissues. Thus, this study identifies the interaction of Erbin and ErbB2 as a novel drug target linking another clinical molecular target, HSP90, in ErbB2-positive breast cancer.

Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation

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In mitophagy, damaged mitochondria recruit parkin to ubiquitinate proteins on the outer mitochondrial membrane, targeting mitochondria for autophagosome engulfment and degradation. However, the proteins involved in mediating autophagosome formation to degrade damaged and ubiquitinated mitochondria remain unknown. We used live cell imaging to demonstrate that optineurin is actively recruited to parkin-labeled ubiquitinated mitochondria and is stabilized by its ubiquitin binding domain. Optineurin binds the autophagosome protein LC3 (microtubule-associated protein light chain 3), and this binding recruits autophagosome assembly around damaged mitochondria. We find (pp. E4439–E4448) that the E478G optineurin mutation, causative for the neurodegenerative disease amyotrophic lateral sclerosis, disrupts autophagosome recruitment. As mutations in parkin are linked to Parkinson's disease, this study indicates that defects in a single mitochondrial degradation pathway lead to neurodegenerative diseases with distinct pathologies.

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Quantitation of interactions between two DNA loops demonstrates loop domain insulation in *E. coli* cells

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Genes are frequently regulated by interactions between proteins that bind to the DNA near the gene and proteins that bind to DNA sites located far away, with the intervening DNA looped out. In eukaryotic genomes, genes and their distant sites are intermingled in complex ways and it is not understood how the correct connections are formed. Using two pairs of DNA-looping sites in bacterial cells, we tested the idea that one DNA loop can either assist or interfere with the formation of another DNA loop. By measuring the strength of these interactions between loops, we showed that this mechanism is capable of directing a distant site to the correct gene and preventing it contacting the wrong gene (pp. E4449–E4457).

Feedback regulation via AMPK and HIF-1 mediates ROS-dependent longevity in *Caenorhabditis elegans*

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Reactive oxygen species (ROS) have long been thought to cause aging and considered to be toxic byproducts generated during mitochondrial respiration. Surprisingly, recent studies show that modestly increased ROS levels lengthen lifespan, at least in the roundworm *Caenorhabditis elegans*. It was unclear how the levels of potentially toxic ROS are regulated and how ROS promote longevity. Here (pp. E4458–E4467) we demonstrate that ROS activate two proteins, AMP-activated kinase (AMPK) and hypoxia-inducible factor 1 (HIF-1), to promote longevity by increasing immunity. Further, we find that internal ROS levels are reduced by AMPK while being amplified by HIF-1 when animals are stimulated to have higher ROS levels. Thus, balancing ROS at optimal levels appears to be crucial for organismal health and longevity.

CHD8 regulates neurodevelopmental pathways associated with autism spectrum disorder in neural progenitors

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Truncating mutation of chromodomain helicase DNA-binding protein 8 (*CHD8*) represents one of the strongest known risk factors for autism spectrum disorder (ASD). We mimicked the effects of such heterozygous loss-of-function mutations in neural progenitor cells and integrated RNA sequencing with genome-wide delineation of CHD8 binding. Our results (pp. E4468–E4477) reveal that the molecular mechanism by which *CHD8* alters neurodevelopmental pathways may involve both direct and indirect effects, the latter involving down-regulation following *CHD8* suppression. We also find that *chd8* suppression in zebrafish results in macrocephaly, consistent with observations in patients harboring loss-of-function mutations. We show that reduced expression of *CHD8* impacts a variety of other functionally distinct ASD-associated genes, suggesting that the diverse functions of ASD risk factors may constitute multiple means of triggering a smaller number of final common pathways.

Human TLR10 is an anti-inflammatory pattern-recognition receptor

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We demonstrate (pp. E4478–E4484) the biological role of TLR10, the only member of the Toll-like receptor (TLR)-family so far without a known function. We show that TLR10 acts as an inhibitory receptor, with suppressive effects. Blocking TLR10 by specific antibodies significantly upregulated TLR2-mediated cytokine production. Additionally, we show that individuals carrying loss-of-function SNPs in TLR10 display upregulation of TLR2-mediated cytokine production. After challenging human TLR10 transgenic mice with TLR2 ligand pam3CSK4 (Pam3Cys), less inflammation could be observed when compared with wild-type mice. Taking these data together, we show that TLR10 is the only pattern-recognition receptor within the TLR family that is able to dampen TLR2 responses, thereby suppressing immune responses through production of IL-1Ra.

Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity

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Alcohol-dependent subjects frequently develop emotional symptoms that contribute to the persistence of alcohol drinking. These subjects are also characterized by gastrointestinal disturbances. In this study, we showed that alcohol-dependent subjects with altered intestinal permeability had also altered gut-microbiota composition and activity and remained with high scores of depression, anxiety, and alcohol craving after a short-term detoxification program. These results are consistent with the existence of a gut–brain axis in alcohol dependence, in which the gut microbiota could alter the gut-barrier function and influence behavior in alcohol dependence. Therefore, this study (pp. E4485–E4493) opens a previously unidentified field of research for the treatment and the management of alcohol dependence, targeting the gut microbiota.

LEOPARD syndrome-associated SHP2 mutation confers leanness and protection from diet-induced obesity

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LEOPARD syndrome (multiple Lentigines, Electrocardiographic conduction abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth, sensorineural Deafness; LS) is a rare genetic disease associating various developmental defects

mainly caused by inactivating mutations of the tyrosine phosphatase SHP2 (Src-homology 2 domain-containing phosphatase 2). SHP2 is a key regulator of essential signaling pathways (MAPK, PI3K), which confer on SHP2 major roles in development and metabolism control. However, nothing is known about the metabolic status of LS. We thus performed an extensive metabolic exploration of an original LS mouse model (pp. E4494–E4503). These mice display a lean phenotype (reduced adiposity, improved carbohydrate metabolism), translating into resistance to obesity and associated disorders upon obesogenic diet. This phenotype correlated with defective adipogenesis, better insulin signaling, and enhanced energy expenditure and was partially corrected by MAPK inhibition. Preliminary data in LS patients are in agreement with these findings.

Identification and characterization of alphavirus M1 as a selective oncolytic virus targeting ZAP-defective human cancers

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Although oncolytic virotherapy is showing great promise in clinical trials, not all patients are benefiting. Identifying predictors of therapeutic effectiveness for each oncolytic virus would provide a good chance to increase response rate. Here (pp. E4504–E4512), we describe an alphavirus (M1) that possesses selective and potent antitumor activity through intravenous infusion, whereas its replication is controlled by the zinc-finger antiviral protein (ZAP) gene. A survey of cancer tissue banks reveals that ZAP is commonly deficient in human cancers, suggesting extensive application prospects of M1. Our work provides an example of a potentially personalized cancer therapy using a targeted oncolytic virus that can be selectively administered to patients with ZAP-deficient tumors. We predict that such agents will form the armamentarium of cancer therapy in the future.

Mapping of transcription factor motifs in active chromatin identifies IRF5 as key regulator in classical Hodgkin lymphoma

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Human lymphomas and leukemias are characterized by molecular and structural alterations of transcription factors (TFs). The identification of such deregulated TFs is therefore central to the understanding of lymphomagenesis. We addressed this question in classical Hodgkin lymphoma (HL), a common B-cell–derived malignancy that is one of the most prominent examples for complex patterns of deregulated TFs including the activation of NF-κB or AP-1 and a profound deregulation of lineage-specific TFs. We found (pp. E4513–E4522) that IRF5 together with NF-κB induces a number of HL characteristic features in non-Hodgkin cells, such as expression of cytokines and chemokines or AP-1 activation. Our work exemplifies how the global lymphoma type-specific characterization of TF activities can improve the understanding of tumor biology.

PGC-1β promotes enterocyte lifespan and tumorigenesis in the intestine

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The mucosa of the small intestine is renewed completely every 3–5 d during the entire lifetime through the continuous steps of proliferation, migration, and differentiation of the cells of the mucosa from the crypt site on the bottom to the villus site on the top of the mucosa. The factors that regulate enterocyte lifespan and aging are of special interest as related to colon cancer susceptibility. Here (pp. E4523–E4531), using genetically modified gain- and loss-of-function models, we present the importance of the mitochondrial respiration chain and reactive oxygen species homeostasis in the gut and identify the protein peroxisome proliferator-activated receptor-γ coactivator-1β as a gene-expression modulator of enterocyte lifespan in both normal and tumoral conditions.

Structural basis of the regulatory mechanism of the plant CIPK family of protein kinases controlling ion homeostasis and abiotic stress

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The transport of ions through the plant cell membrane establishes the key physicochemical parameters for cell function. Stress situations such as those created by soil salinity or low potassium conditions alter the ion transport across the membrane producing dramatic changes in the cell turgor, the membrane potential, and the intracellular pH and concentrations of toxic cations such as sodium and lithium. As a consequence, fundamental metabolic routes are inhibited. The CIPK family of 26 protein kinases regulates the function of several ion transporters at the cell membrane to restore ion homeostasis under stress situations. Our analyses (pp. E4532–E4541) provide an explanation on how the CIPKs are differentially activated to coordinate the adequate cell response to a particular stress.