PNAS Plus Significance Statements

The search engine manipulation effect (SEME) and its possible impact on the outcomes of elections

Robert Epstein and Ronald E. Robertson

We present evidence from five experiments in two countries suggesting the power and robustness of the search engine manipulation effect (SEME). Specifically, we show that (*i*) biased search rankings can shift the voting preferences of undecided voters by 20% or more, (*ii*) the shift can be much higher in some demographic groups, and (*iii*) such rankings can be masked so that people show no awareness of the manipulation. Knowing the proportion of undecided voters in a population who have Internet access, along with the proportion of those voters who can be influenced using SEME, allows one to calculate the win margin below which SEME might be able to determine an election outcome. (See pp. E4512–E4521.)

Systematic review of current efforts to quantify the impacts of climate change on undernutrition

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The World Health Organization and the Intergovernmental Panel for Climate Change propose undernutrition as the most significant impact of climate change on child health. The question then arises: Where does the empirical evidence to back this claim come from? Current evidence for the impacts of climate on childhood undernutrition draws on a limited number of heterogeneous studies with methodological limitations and is based predominantly on secondary data. Establishing and validating causal pathways among complex confounding factors remain the main challenge in quantifying the climate-attributable fraction of undernutrition. Systematically generating evidence from long-term, high-quality primary data on a range of factors (agricultural, environmental, socioeconomic, and health) at the household level is critical for designing adaptation strategies, particularly for subsistence farmers. (See pp. E4522–E4529.)

Creative template-dependent synthesis by human polymerase mu

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Template-dependent DNA polymerases usually add nucleotides to the 3′ end of a primer, using the first available template-strand nucleotide as a guide. This behavior holds true for all polymerases, except one, DNA polymerase μ. When presented with 2-nt single- or double-strand gaps, polymerase μ (Pol μ) engages the substrate with the last available template-strand nucleotide closest to the 5′-phosphate on the downstream end of the gap, guiding synthesis. Crystal structures of Pol μ with a 2-nt gapped DNA substrate explain how the unpaired base is accommodated in the active site, and yield insights into the behavior of this polymerase within the context of nonhomologous end joining in DNA double-strand break repair. (See pp. E4530–E4536.)

Essential role for polymerase specialization in cellular nonhomologous end joining

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Nonhomologous end joining (NHEJ) is a DNA double-strand break repair pathway required for development of the adaptive immune response, maintenance of cellular proliferative capacity, and the response to several commonly used cancer treatments. A major challenge faced by this pathway is that chromosome breaks can have dirty end structures, making them difficult to repair. We show here that two mammalian DNA polymerases have an unexpectedly pivotal role in helping resolve such ends. Each is proficient in different contexts and has a differing impact on repair fidelity. This work sheds light on how NHEJ has evolved to be flexible during repair and identifies two polymerases as critical for this process. (See pp. E4537–E4545.)

Dynamic localization of Mps1 kinase to kinetochores is essential for accurate spindle microtubule attachment

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The spindle assembly checkpoint (SAC) works as a surveillance mechanism to ensure accurate segregation of genetic materials during cell division. Protein kinase monopolar spindle 1 (Mps1) plays a key role in SAC, but the mechanism of Mps1 action in chromosome segregation remains elusive. In this study, we identified a previously undefined structural determinant of Mps1 named "IRK" (internal region for kinetochore localization) and demonstrated its functional importance in accurate kinetochore–microtubule attachment. Mechanistically, a dynamic hierarchical interaction between Mps1 and the nuclear division cycle 80 complex (Ndc80C) orchestrates accurate mitosis, because persistent association of inactive Mps1 with Ndc80C via the IRK perturbs correct kinetochore–microtubule attachment. Our results provide a new mechanistic insight into the spatiotemporal dynamics of Mps1 activity at the kinetochore in mitosis. (See pp. E4546–E4555.)

Ablation of XP-V gene causes adipose tissue senescence and metabolic abnormalities

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The metabolic syndrome has evolved to be a major health issue globally. The association between genome integrity and metabolic abnormalities is not well understood. Our results indicate that increased DNA damage and persistent activation of the DNA damage response induce adipocyte senescence in DNA polymerase η knockout (*pol* $\eta^{-/-}$) mice. Suppression of adipocyte senescence with a p53

inhibitor, pifithrin-α, alleviated metabolic abnormalities in *pol* $\eta^{-/-}$ mice. An increase or decrease in DNA damage affected the senescence status of adipocytes accordingly, which was also in concordance with the severity of metabolic abnormalities in the *pol* $\eta^{-/-}$ mice. Our current results indicate that reduced genome integrity plays a causative role in provoking adipocyte senescence that leads to development of obesity and insulin resistance. (See pp. E4556–E4564.)

Fork rotation and DNA precatenation are restricted during DNA replication to prevent chromosomal instability

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Genome inheritance requires the complete resolution of all intertwines within parental DNA. This is facilitated by fork rotation and precatenation of the newly replicated DNA. However, the general importance and frequency of fork rotation in vivo are poorly understood. We find that the evolutionarily conserved Timeless and Tipin proteins actively inhibit fork rotation in budding yeast. In their presence, fork rotation appears restricted to hard-to-replicate fragile sites. In their absence, excessive fork rotation leads to damage accumulating in the replicated sister chromatids, especially at known yeast fragile sites. Therefore, fork rotation appears to be restricted to contexts where it is absolutely required for unwinding, and this restriction is required to prevent precatenation inducing excessive chromosomal fragility. (See pp. E4565–E4570.)

Intrinsic mutagenic properties of 5-chlorocytosine: A mechanistic connection between chronic inflammation and cancer

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Chronic inflammation is a significant risk factor for cancer and other human diseases. During chronic inflammation, cells exposed to neutrophil-derived hypochlorous acid accumulate in their genomes the DNA lesion 5-chlorocytosine (5ClC). Using a battery of chemical, structural, and genetic tools, the present study demonstrates that 5ClC is a mutagenic lesion, suggesting that genomic 5ClC accumulation could have very serious biological consequences. 5ClC induces C→T transitions, a type of mutation commonly observed in tissues under inflammatory stress as well as in the genomes of inflammation-driven cancers. Thus, the mutagenic properties of 5ClC represent an appealing molecular mechanism by which chronic inflammation induces the genetic changes that potentially enable and stimulate carcinogenesis. (See pp. E4571–E4580.)

RabGDIα is a negative regulator of interferon-γ–inducible GTPase-dependent cell-autonomous immunity to *Toxoplasma gondii*

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IFN-γ is a proinflammatory cytokine and stimulates induction of ∼2,000 genes, including IFN-γ–inducible GTPases, such as immunity-related GTPases (IRGs) and guanylate-binding proteins (GBPs), that are critically required for cell-autonomous host defense against the vacuolar pathogen *Toxoplasma gondii*. Mechanisms of how recruitment of these GTPases to the vacuoles is positively regulated have been gradually elucidated. However, the negative regulation remains unknown. Here, we show that Rab GDP dissociation inhibitor α (RabGDIα) acts as a suppressor of IFN-γ–inducible GTPases, such as Gbp2 and Irga6. RabGDIα deficiency resulted in enhanced IFN-γ–mediated *T. gondii* clearance in vitro and in vivo. Furthermore, RabGDIα inhibited the act of Gbp2 and Irga6 through the lipid-binding pocket. Thus, our current study demonstrates a negative regulatory mechanism for IFN-γ–inducible GTPase-dependent cell-autonomous immunity. (See pp. E4581–E4590.)

Aldosterone-stimulating somatic gene mutations are common in normal adrenal glands

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Primary aldosteronism (PA) represents the most common adrenal disease and cause of secondary hypertension. However, little is known regarding adrenal cellular origins. Recently, subcapsular aldosterone-producing cell clusters (APCCs) were observed in normal adrenals. We hypothesize that APCCs are a contributor to PA. Here, we characterized the APCC transcriptome and show that *CYP11B2* expression is increased compared with the rest of the adrenal cortex. We also show that many APCCs harbor known aldosterone-producing adenoma (APA)-related ion channels/pumps (*ATPase, Na^+/K^+ transporting, α1-polypeptide* and *calcium channel, voltage-dependent, L-type, α1D-subunit*) mutations that stimulate *CYP11B2* expression and aldosterone production. Importantly, the mutation spectrum seen in APCCs differs from that reported for APA. These results provide molecular support for APCC as a precursor of PA. (See pp. E4591–E4599.)

Chemotherapy triggers HIF-1–dependent glutathione synthesis and copper chelation that induces the breast cancer stem cell phenotype

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We demonstrate that glutathione biosynthesis is controlled by hypoxia-inducible factor 1 and is critical for chemotherapy-induced enrichment of breast cancer stem cells, making it an attractive therapeutic target in triple-negative breast cancer, which is the only subset of breast cancers for which there is no available targeted therapy. We also delineate a molecular mechanism in which glutathione functions as a signaling molecule to activate the breast cancer stem cell phenotype, establishing cross-talk between cancer metabolism and signal transduction. We also demonstrate that mitogen-activated protein kinase kinase (MEK)-ERK inhibitors and copper chelators have the countertherapeutic effect of inducing breast cancer stem cell enrichment. (See pp. E4600–E4609.)

FXR1P is a GSK3β substrate regulating mood and emotion processing

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This study provides a cellular mechanism for the regulation of behavioral dimensions pertinent to mood disorders. Inhibition of glycogen synthase kinase 3β (GSK3β) is a shared action of drugs used for bipolar disorder. However, the substrates through which this kinase regulates mood are not known. We identified fragile X mental retardation-related protein 1 (FXR1P) as a substrate for GSK3β. Phosphorylation of FXR1P by GSK3β would lead to its down-regulation. Overexpression of FXR1P in the mouse prelimbic cortex elicits behavioral responses comparable to those of drugs inhibiting GSK3β. Furthermore, functional gene polymorphisms affecting FXR1P or GSK3β gene expression interact to regulate emotional brain responsiveness and stability in humans. These observations indicate that regulation of FXR1P by GSK3β contributes to regulating mood and emotion processing. (See pp. E4610–E4619.)

Perceptual transparency from image deformation

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The perception of liquids, particularly water, is a vital sensory function for survival, but little is known about the visual perception of transparent liquids. Here we show that human vision has excellent ability to perceive a transparent liquid solely from dynamic image deformation. No other known image cues are needed for the perception of transparent surfaces. Static deformation is not effective for perceiving transparent liquids. Human vision interprets dynamic image deformation as caused by light refraction at the moving liquid's surface. Transparent liquid is well perceived from artificial image deformations, which share only basic flow features with image deformations caused by physically correct light refraction. (See pp. E4620–E4627.)