PNAS Plus Significance Statements

Prestimulus oscillatory power and connectivity patterns predispose conscious somatosensory perception

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Experimental use of near-threshold stimuli is regarded as an important approach to study neural processes leading to conscious access (defined in operational terms as "reportability"). Looking to what extent prestimulus periods contribute to the variability of perceptual states has become increasingly popular, frequently pointing to an increased excitability of relevant sensory regions. Here (pp. E417–E425), we aim to extend this "local" perspective by a network approach. A framework called "Windows to Consciousness" is introduced. We postulate that along with an enhanced excitability, preestablished pathways of information flow are a crucial ingredient, determining whether an upcoming near-threshold stimulus will be consciously perceived. Using magnetoencephalography combined with state-of-the-art source-imaging approaches, we are able to report supportive evidence.

Structures of yeast mitochondrial ADP/ATP carriers support a domain-based alternating-access transport mechanism

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ADP/ATP carriers are archetypal members of the mitochondrial carrier family of transport proteins, which are thought to operate by a common but unresolved mechanism. Members of this family play key roles in many aspects of cell physiology and are implicated in several severe human diseases. Here, we present the structures of Aac2p and Aac3p, ADP/ATP carriers from *Saccharomyces cerevisiae*, determined by X-ray crystallography. Together with mutagenesis and functional assays (pp. E426–E434), the structures support an alternating-access transport mechanism involving domain-based motions, where salt-bridge networks act as gates, providing access to a central substrate-binding site.

Discrete mechanisms of mTOR and cell cycle regulation by AMPK agonists independent of AMPK

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Cancer cells reprogram their metabolism for optimal growth and survival. AMPK-activated protein kinase (AMPK) is a key energy sensor that controls many metabolic pathways including metabolic reprogramming. However, its role in cancer is poorly understood. Some studies claim that it has a tumor suppressor role while others show its protumor role. Two AMPK-activating compounds (including metformin, now in many clinical trials) are widely used to suppress cancer cell proliferation. We found (pp. E435–E444) that AMPK is abundantly expressed in high-grade gliomas and, in contrast to popular belief, these two AMPK activators suppressed glioma cell proliferation through unique AMPK-independent mechanisms.

Integrated description of protein dynamics from room-temperature X-ray crystallography and NMR

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Most proteins are inherently flexible and their dynamics play a central role in their biological functions. A molecular level understanding of protein function and mechanism requires an accurate description of the atomic coordinates in both time and space. Here we show (pp. E445–E454), through studies of the enzyme dihydrofolate reductase, that multiconformer models derived from room-temperature X-ray crystallographic data can be used synergistically with nuclear magnetic resonance relaxation measurements to provide a detailed description of both the amplitude and timescale of fluctuations in atomic coordinates. This hybrid approach provides a more complete description of protein dynamics than can be obtained from either method alone. The room-temperature crystallographic ensemble accurately reflects the picosecond–nanosecond motions of the protein backbone and side chains.

Searching for missing heritability: Designing rare variant association studies

Or Zuk, Stephen F. Schaffner, Kaitlin Samocha, Ron Do, Eliana Hechter, Sekar Kathiresan, Mark J. Daly, Benjamin M. Neale, Shamil R. Sunyaev, and Eric S. Lander

Discovering the genetic basis of common diseases, such as diabetes, heart disease, and schizophrenia, is a key goal in biomedicine. Genomic studies have revealed thousands of common genetic variants underlying disease, but these variants explain only a portion of the heritability. Rare genetic variants are also likely to play an important role, but few examples are known thus far, and initial discovery efforts with small sample sizes have had only limited success. In this paper (pp. E455–E464), we describe an analytical framework for the design of rare variant association studies of disease. It provides guidance with respect to sample size, as well as the use of disruptive and missense alleles, allele frequency thresholds, isolated populations, gene sets, and coding vs. noncoding regions.

Thymocyte apoptosis drives the intrathymic generation of regulatory T cells

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Thymus-derived regulatory T cells (tTregs) are vital to maintaining immune homeostasis, and as such the signals driving their development have been extensively studied. Despite this, a cohesive model describing tTreg generation and aligning all conflicting data has been elusive. Here (pp. E465–E473) we outline a comprehensive model controlling the generation of tTregs and show that tTreg generation is tied to thymic apoptosis. We show that the presence of apoptotic cells in the thymus drives the production of TGFβ intrathymically and that this cytokine

then acts to specify the tTreg fate. Thus, our results reveal an apoptosis–TGFβ–Foxp3 axis that mediates the development of tTregs.

Peptides genetically selected for NF-κB activation cooperate with oncogene Ras and model carcinogenic role of inflammation

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Despite general acceptance of the link between chronic inflammation and cancer, the precise molecular mechanisms underlying the cancer-promoting effects of inflammation remain undefined. Inducible transcription factor NF-κB is the key regulator of inflammation, which is commonly deregulated in cancer cells to become constitutively active. Whether this deregulation contributes to malignant transformation was the main question addressed in this study. We isolated a series of genetic elements encoding artificial intracellular proteins capable of constitutive activation of NF-κB, named NF-κB–activating selectable peptides (NASPs), and demonstrated that all of them had carcinogenic activity in conventional cellular models (pp. E474–E483). Specifically, NASPs made normal rodent cells susceptible to malignant transformation by oncogene Ras, which cannot do it on its own. This result defines chronically active NF-κB as an oncogene.

RIG-I–like receptor LGP2 protects tumor cells from ionizing radiation

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An undesirable outcome of radiotherapy (ionizing radiation, IR) of cancer is the emergence of radioresistant cells. We report (pp. E484–E491) that Laboratory of Genetics and Physiology 2 (LGP2), a resident RIG-I (retinoic acid inducible gene I)–like receptor protein, can induce radioresistance. IR induces interferon and stimulates accumulation of LGP2. In turn, LGP2 shuts off the synthesis of interferon and blocks its cytotoxic effects. Ectopic expression of LGP2 enhances resistance to IR, whereas depletion enhances cytotoxic effects of IR. Here we show that LGP2 is associated with radioresistance in numerous diverse cancer cell lines. Examination of available databases links expression of LGP2 with poor prognosis in cancer patients.

Multiple pathways for *Plasmodium* ookinete invasion of the mosquito midgut

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Malaria is among the most devastating parasitic diseases. Invasion of the mosquito midgut by motile malaria ookinetes requires specific interactions between proteins of both organisms. This study (pp. E492–E500) reports on a novel mosquito midgut receptor [enolase-binding protein (EBP)] that is recognized by an ookinete surface ligand (enolase). We also show that *Plasmodium* ookinetes invade the mosquito midgut by at least two different pathways: one dependent on and the other independent of the EBP–enolase interaction. Furthermore, we provide evidence for a second universal midgut receptor essential for midgut invasion by both human and rodent malaria parasites. These findings may lead to the development of novel targets for transmission-blocking interventions.

A variable homopolymeric G-repeat defines small RNA-mediated posttranscriptional regulation of a chemotaxis receptor in *Helicobacter pylori*

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To establish long-term infections, bacterial pathogens must adapt their gene expression through sensing and responding to changing conditions or selection of genotypic variations within the population. Hypervariable simple sequence repeats (SSRs) in coding sequences or promoters are a major source of phase variation and often associated with genes involved in host interaction. While their impact on gene regulation at the DNA level is established, we now demonstrate a connection between SSRs and small RNA (sRNA)-mediated post-transcriptional regulation. We show (pp. E501–E510) that a homopolymeric G-repeat within the leader of a chemotaxis receptor mRNA in *Helicobacter pylori* is directly targeted by a small RNA. The length of this G-repeat varies among different *Helicobacter* strains and thereby determines sRNA-mediated translational repression or activation and strain-specific regulation.

Strain-specific innate immune signaling pathways determine malaria parasitemia dynamics and host mortality

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Malaria infection causes a severe disease with diverse symptoms. The molecular mechanisms underlying the differences of malaria pathology remain unknown or controversial. Here we infected mice with two closely related strains of rodent malaria parasite *Plasmodium yoelii* and characterized host genome-wide responses to the infections. We found that in mice infected with parasite N67, type I interferon was produced to a high level, leading to suppression of parasitemia. We further characterized the molecular mechanisms and identified host receptors in recognizing parasite ligands. In contrast, mice infected with N67C parasite mounted a strong inflammatory response, leading to severe pathology and host death. This study (pp. E511–E520) reveals previously unrecognized mechanisms associated with strain-specific malaria infection and provides important information for studying human malaria pathogenesis.

Lateral organ boundaries 1 is a disease susceptibility gene for citrus bacterial canker disease

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Citrus bacterial canker, which is caused by several species in the genus *Xanthomonas*, is a severe disease with worldwide distribution affecting all the commercially important citrus species and cultivars. The mechanisms of canker development, involving erumpent pustule formation and bacterial growth, are not known. Our findings (pp. E521–E529) suggest that virulence determinants in several pathogens activate a single host disease susceptibility (S) gene that has a critical contribution to bacterial growth and host pustule development. The S gene represents an excellent candidate for control measures for the citrus bacterial canker.