PNAS Plus Significance Statements

Allosteric activation of apicomplexan calcium-dependent protein kinases

Jessica R. Ingram, Kevin E. Knockenhauer, Benedikt M. Markus, Joseph Mandelbaum, Alexander Ramek, Yibing Shan, David E. Shaw, Thomas U. Schwartz, Hidde L. Ploegh, and Sebastian Lourido

The activation of kinases by Ca^{2+} represents a vital class of signaling interactions that regulates many biological processes. The mechanism of activation for these enzymes is conserved and characterized by removal of an inhibitory element from the kinase domain. We report a previously unidentified mechanism for the activation of essential apicomplexan calcium-dependent protein kinases (CDPKs). Using *Toxoplasma* CDPK1 as a representative, we demonstrate that the kinase domain is intrinsically inactive and requires stabilization for activity. This distinct mechanism of activation reveals a susceptibility in CDPKs, which we exploit to effectively inhibit them. When viewed in the context of the entire protein kinase family, our results emphasize the remarkable adaptability of the kinase fold to diverse forms of regulation. (See pp. E4975–E4984.)

The COUP-TFII/Neuropilin-2 is a molecular switch steering diencephalon-derived GABAergic neurons in the developing mouse brain

Shigeaki Kanatani, Takao Honda, Michihiko Aramaki, Kanehiro Hayashi, Ken-ichiro Kubo, Mami Ishida, Daisuke H. Tanaka, Takeshi Kawauchi, Katsutoshi Sekine, Sayaka Kusuzawa, Takahiko Kawasaki, Tatsumi Hirata, Hidenori Tabata, Per Uhlén, and Kazunori Nakajima

Recently the preoptic area (POa) has been shown to be a source of GABAergic neurons in the medial amygdala and cerebral cortex, where they are thought to play a pivotal role in emotions and intelligence, respectively. However, it is unknown how the POa-derived neurons migrate and selectively segregate into either the amygdala or cortex. By using focal in utero labeling of the POa, we show that switching on/off the transcription factor COUP-TFII (Chicken ovalbumin upstream promoter transcription factor II) and the receptor Neuropilin-2 (Nrp2) directs the POa-derived neurons toward either the amygdala or cortex. Our study revealed an essential role of COUP-TFII/Nrp2 expression dynamics in the development of the amygdala and cortex. (See pp. E4985–E4994.)

Positive feedback between RNA-binding protein HuD and transcription factor SATB1 promotes neurogenesis

Feifei Wang, Joseph J. Tidei, Eric D. Polich, Yu Gao, Huashan Zhao, Nora I. Perrone-Bizzozero, Weixiang Guo, and Xinyu Zhao

RNA-binding proteins play central roles in posttranscriptional gene regulation. HuD is one of the first markers used for neuronal lineage; however, the function of HuD in neural stem cell differentiation is largely unexplored. In addition, although it has been shown that *HuD* mRNA levels increase during neuronal differentiation, to date few studies have examined the mechanism controlling the expression of HuD during neural differentiation. In this study, we investigated the role of HuD in neural stem cell differentiation and uncovered an underlying molecular mechanism. Our results unveil a novel positive feedback network between an RNA-binding protein and a transcription factor that plays critical regulatory roles during neuronal differentiation. (See pp. E4995–E5004.)

Chemical fingerprints encode mother–offspring similarity, colony membership, relatedness, and genetic quality in fur seals

Martin A. Stoffel, Barbara A. Caspers, Jaume Forcada, Athina Giannakara, Markus Baier, Luke Eberhart-Phillips, Caroline Müller, and Joseph I. Hoffman

Understanding olfactory communication in natural vertebrate populations requires knowledge of how genes and the environment influence highly complex individual chemical fingerprints. To understand how relevant information is chemically encoded and may feed into mother–offspring recognition, we therefore generated chemical and genetic data for Antarctic fur seal mother–pup pairs. We show that pups are chemically highly similar to their mothers, reflecting a combination of genetic and environmental influences. We also reveal associations between chemical fingerprints and both genetic quality and relatedness, the former correlating positively with substance diversity and the latter encoded mainly by a small subset of substances. Dissecting apart chemical fingerprints to reveal subsets of potential biological relevance has broad implications for understanding vertebrate chemical communication. (See pp. E5005–E5012.)

Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping

Rakesh Santhanam, Van Thi Luu, Arne Weinhold, Jay Goldberg, Youngjoo Oh, and Ian T. Baldwin

Plant roots associate with the diverse microbial community in soil and can establish mutualistic relationships with microbes. The genetic characterization of the plant microbiome (total microbiota of plants) has intensified, but we still lack experimental proof of the ecological function of the root microbiome. Without such an understanding, the use of microbial communities in sustainable agricultural practices will be poorly informed. Through continuous cropping of a seed-sterilized native plant, we inadvertently recapitulated a common agricultural dilemma: the accumulation of phytopathogens. Experimental inoculations of seeds with native bacterial consortium during germination significantly attenuated plant mortality, demonstrating that a plant's opportunistic mutualistic associations with soil microbes have the potential to increase the resilience of crops. (See pp. E5013–E5020.)

Fate of a mutation in a fluctuating environment

Ivana Cvijović, Benjamin H. Good, Elizabeth R. Jerison, and Michael M. Desai

Evolution in variable environments depends crucially on the fates of new mutations in the face of fluctuating selection pressures. In constant environments, the relationship between the selective effect of a mutation and the probability that it will eventually fix or go extinct is well understood. However, our understanding of fixation probabilities in fluctuating environmental conditions is limited. Here, we show that temporal fluctuations in environmental conditions can have dramatic effects on the fate of each new mutation, reducing the efficiency of natural selection and increasing the fixation probability of all mutations, including those that are strongly deleterious on average. This makes it difficult for a population to maintain specialist adaptations, even if their benefits outweigh their costs. (See pp. E5021–E5028.)

Burkholderia bacteria infectiously induce the proto-farming symbiosis of *Dictyostelium* amoebae and food bacteria

Susanne DiSalvo, Tamara S. Haselkorn, Usman Bashir, Daniela Jimenez, Debra A. Brock, David C. Queller, and Joan E. Strassmann

Symbionts can provide hosts with many advantages including defensive capabilities and novel nutrients. However, symbionts may begin as pathogens that only subsequently become beneficial. In the *Dictyostelium discoideum* farming symbiosis some amoebas stably associate with bacterial partners. We demonstrate that amoeba-associated *Burkholderia* can initiate a farming symbiosis with naive amoeba hosts. *Burkholderia* decreases amoeba spore productivity in food-rich conditions but, because of the induction of bacterial food carriage, sometimes increases spore productivity in food-scarce conditions. Detrimental effects of *Burkholderia* colonization differ among *Burkholderia* genotypes and, in some cases, between new and old amoeba hosts, suggesting some coevolution within the association. These results suggest that *Burkholderia* exerts both pathogenic and mutualistic effects on its host in conditionally dependent ways. (See pp. E5029–E5037.)

IFN-γ ameliorates autoimmune encephalomyelitis by limiting myelin lipid peroxidation

Rebecca A. Sosa, Cathi Murphey, Rachel R. Robinson, and Thomas G. Forsthuber

The mechanisms guiding tissue repair in autoimmune-mediated diseases of the CNS are not fully understood, but cytokines are believed to play an important role. The proinflammatory cytokine IFN-γ has been implicated in the pathogenesis of multiple sclerosis and its animal model, experimental autoimmune encephalomyelitis (EAE). Paradoxically, knockout studies in mice showed that EAE is exacerbated when the cytokine's signaling is disrupted, but its mechanism of protection has not been resolved. Here, we show a critical role for IFN-γ in activating antigen-presenting cells toward a protective phenotype in the CNS by removing extracellular myelin debris and limiting the availability of substrate for oxidative stress-generated neurotoxic lipid peroxidation products. (See pp. E5038–E5047.)

Oligoribonuclease is the primary degradative enzyme for pGpG in *Pseudomonas aeruginosa* that is required for cyclic-di-GMP turnover

Mona W. Orr, Gregory P. Donaldson, Geoffrey B. Severin, Jingxin Wang, Herman O. Sintim, Christopher M. Waters, and Vincent T. Lee

Cyclic-di-GMP (c-di-GMP) is a ubiquitous bacterial second messenger that regulates complex behaviors such as biofilm formation. These behaviors are changed by altering the intracellular concentration of c-di-GMP. Degradation of c-di-GMP occurs by a two-step process in which one set of phosphodiesterases (PDE-As) linearize the molecule into 5′-phosphoguanylyl-(3′,5′)-guanosine (pGpG), followed by hydrolysis by unidentified phosphodiesterases (PDE-Bs) into two GMPs. High levels of pGpG inhibit PDE-As, and thus PDE-B activity is important in maintaining c-di-GMP homeostasis. However, the identity of the PDE-B(s) remained unknown. Using a high-throughput binding screen, we identify oligoribonuclease (Orn) as a putative PDE-B. We demonstrate that Orn is the primary source of PDE-B activity in *Pseudomonas aeruginosa*. Identification of Orn as the primary PDE-B completes the c-di-GMP signaling pathway. (See pp. E5048–E5057.)

Subset of early radial glial progenitors that contribute to the development of callosal neurons is absent from avian brain

Fernando García-Moreno and Zoltán Molnár

Understanding development and evolution of the neocortex has important implications. We describe here a major difference between avian and mammalian dorsal pallial progenitors regarding their fate restrictions. In mouse cortex we identified an early population of radial glial cells that are delayed in the generation of neurons. After self-renewal and transit-amplifying mitoses, these murine progenitors become committed to the genesis of upper-layer callosal pyramidal neurons and glia. In the chick embryonic pallium we identified a homologous population of progenitors; however, these have no delayed neurogenesis and their lineage is therefore not segregated from that of the other pallial progenitors. We hypothesize on the relation between the early neurogenic delay of progenitors and the origin of the corpus callosum. (See pp. E5058–E5067.)

Transcription factor p63 controls the reserve status but not the stemness of horizontal basal cells in the olfactory epithelium

Nikolai Schnittke, Daniel B. Herrick, Brian Lin, Jesse Peterson, Julie H. Coleman, Adam I. Packard, Woochan Jang, and James E. Schwob

Harnessing the inherent capability of stem cells to maintain and regenerate injured tissues is a prerequisite for their use in mending damage to the nervous system. In the olfactory epithelium stem cells accomplish neurogenesis and epithelial repair throughout life to an extent not seen elsewhere in the nervous system. Here we show that the transcription factor protein 63 (*p63*) is a master regulator of the transition from the reserve to the active stem cell pool in the epithelium. Loss of *p63* expression in reserve basal cells is necessary and sufficient for activation, without compromising stem cell status. Identification of this central mechanism provides a target for stem cell activation in this uniquely accessible source of patient-specific, neurogenic stem cells. (See pp. E5068–E5077.)

Synapse-specific IL-1 receptor subunit reconfiguration augments vulnerability to IL-1β in the aged hippocampus

G. Aleph Prieto, Shikha Snigdha, David Baglietto-Vargas, Erica D. Smith, Nicole C. Berchtold, Liqi Tong, Dariush Ajami, Frank M. LaFerla, Julius Rebek Jr., and Carl W. Cotman

There is a growing understanding that inflammation impairs synaptic plasticity and cognition and that the aged brain has an elevated sensitivity to cognitive impairment by the proinflammatory cytokine interleukin 1β (IL-1β). IL-1β activates different pathways via AcP (proinflammatory) or AcPb (prosurvival) IL-1 receptor subunits. This study demonstrates that the IL-1 receptor subunit system undergoes an age-dependent reconfiguration in hippocampal synapses. This previously undescribed reconfiguration, characterized by an increase in the AcP/AcPb ratio, is responsible for potentiating impairments of synaptic plasticity and memory by IL-1β. Our data reveal a previously unidentified mechanism that explains the age-related vulnerability of hippocampal function to impairment by inflammation and adds another dimension beyond glia to understanding how inflammation causes cognitive decline in aging. (See pp. E5078–E5087.)

RGS9-2–controlled adaptations in the striatum determine the onset of action and efficacy of antidepressants in neuropathic pain states

Vasiliki Mitsi, Dimitra Terzi, Immanuel Purushothaman, Lefteris Manouras, Sevasti Gaspari, Rachael L. Neve, Maria Stratinaki, Jian Feng, Li Shen, and Venetia Zachariou

Neuropathic pain is a complex disorder, characterized by affective and sensory symptoms. Efficient treatment of this condition should target both pain-modulating pathways and mood/affect networks. We show that tricyclic antidepressants (TCAs), which modulate spinal pain processing, also act in the brain reward center to alleviate allodynia and depression-like behaviors. We reveal how one key protein of nucleus accumbens (NAc)-specific signaling affects several molecules/pathways with emerging roles in antinociceptive and antidepressant mechanisms. Our study provides information about the cellular adaptations induced by TCAs in the NAc and novel targets for pain treatment. (See pp. E5088–E5097.)

Uterine activin receptor-like kinase 5 is crucial for blastocyst implantation and placental development

Jia Peng, Diana Monsivais, Ran You, Hua Zhong, Stephanie A. Pangas, and Martin M. Matzuk

Although many studies have yielded tremendous insights into the roles of TGF-β superfamily signaling pathways in physiological and pathophysiological processes, the in vivo roles of TGF-β signaling pathways in many aspects of reproduction remain largely unknown. To address these functions in females, we conditionally deleted the TGF-β type 1 receptor (activin receptor-like kinase 5, ALK5) and demonstrated that absence of TGF-β signaling through ALK5 in the uterus leads to striking abnormalities at different stages of pregnancy, including delayed implantation, disorganization of the trophoblast cells, significantly fewer uterine natural killer cells, and defects in spiral artery remodeling. Our findings provide a mouse model to investigate TGF-β signaling in reproduction and pave the way toward a better understanding of the pathogenesis of pregnancy-related complications in women. (See pp. E5098–E5107.)