PNAS Plus Significance Statements

Peptide transporter DtpA has two alternate conformations, one of which is promoted by inhibitor binding

Christian A. Bippes, Lin Ge, Marcel Meury, Daniel Harder, Zöhre Ucurum, Hannelore Daniel, Dimitrios Fotiadis, and Daniel J. Müller

Proton-dependent oligopeptide transporters are attractive candidates for drug research. To understand their functional modulation by drugs, we applied (pp. E3978–E3986) single-molecule force spectroscopy and characterized how peptide transport facilitated by the dipeptide and tripeptide permease A (DtpA) from *Escherichia coli* is inhibited. In the unbound state DtpA embedded in the physiologically relevant membrane adopts two alternate conformations, which differ mainly in whether the transmembrane α-helix TMH2 is stabilized. TMH2 contains residues that are important for ligand binding and substrate affinity. Inhibitor (Lys[Z-NO_2]-Val) binding to DtpA significantly strengthens the interactions stabilizing TMH2 and guides DtpA to populate the inhibited conformation.

A gating mechanism of pentameric ligand-gated ion channels

Nicolas Calimet, Manuel Simoes, Jean-Pierre Changeux, Martin Karplus, Antoine Taly, and Marco Cecchini

Pentameric ligand-gated ion channels (pLGICs) control membrane conductance in living systems from bacteria to humans. Molecular dynamics simulations based on the structures of the prokaryotic channels from *Gloeobacter violaceus* (GLIC) and *Erwinia chrysanthemi* (ELIC) and the eukaryotic channel from *Caenorhabditis elegans* (GluCl) show that the open-to-closed transition begins with a major quaternary (twisting) transition which is followed by tertiary relaxation of the pore-forming helices. The latter is initiated by the outward tilting of the extracellular β-sandwiches in response to agonist unbinding. The proposed atomic resolution mechanism for channel gating (pp. E3987–E3996), which is in accord with the Monod–Wyman–Changeux model of allostery, is expected to be generally applicable to pLGICs.

p21-mediated RNR2 repression restricts HIV-1 replication in macrophages by inhibiting dNTP biosynthesis pathway

Awatef Allouch, Annie David, Sarah M. Amie, Hichem Lahouassa, Loïc Chartier, Florence Margottin-Goguet, Françoise Barré-Sinoussi, Baek Kim, Asier Sáez-Cirión, and Gianfranco Pancino

Macrophages, with $CD4^+$ T lymphocytes, are a major cell target for HIV-1 infection. We have previously reported that the induction of a cellular protein, the cyclin-dependent kinase p21, inhibits HIV-1 replication in macrophages. We now show (pp. E3997–E4006) that p21 impairs the reverse transcription of HIV-1 and other primate lentiviruses, including the simian immunodeficiency virus (SIV)mac, by blocking the synthesis of cellular deoxynucleotides (dNTP) that are used by retroviral reverse transcriptase for viral DNA synthesis. p21 represses the expression of a key enzyme of the dNTP biosynthesis pathway, the RNR2 subunit of the ribonucleotide reductase. Our findings point to new potential cellular targets for antiretroviral strategies.

Calpain-dependent cytoskeletal rearrangement exploited for anthrax toxin endocytosis

Sun-Young Jeong, Mikhail Martchenko, and Stanley N. Cohen

Bacillus anthracis produces protein toxins that present a major biodefense challenge. Host genes exploited for toxin entry by pathogens are potential targets for therapies that circumvent resistance-inducing pathogen mutations. We report (pp. E4007–E4015) that (*i*) anthrax lethal toxin enters macrophages by associating with higher-order actin filaments that provide the dynamic force for membrane invagination during endocytic trafficking of integrin-containing focal adhesion complexes (FACs) and (*ii*) calpain-mediated cleavage of talin-1, which anchors FACs at the cell surface, facilitates such exploitation. Our findings elucidate steps of anthrax toxin endocytosis and identify a potential target for mitigation of toxicity.

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Deficits in dopaminergic transmission precede neuron loss and dysfunction in a new Parkinson model

Stephanie Janezic, Sarah Threlfell, Paul D. Dodson, Megan J. Dowie, Tonya N. Taylor, Dawid Potgieter, Laura Parkkinen, Steven L. Senior, Sabina Anwar, Brent Ryan, Thierry Deltheil, Polina Kosillo, Milena Cioroch, Katharina Wagner, Olaf Ansorge, David M. Bannerman, J. Paul Bolam, Peter J. Magill, Stephanie J. Cragg, and Richard Wade-Martins

Elevated expression of the presynaptic protein α-synuclein underlies familial and sporadic Parkinson disease (PD). However, our understanding of how increases in α-synuclein levels drive the sequence of events leading to PD is incomplete. Here (pp. E4016–E4025), we apply a multidisciplinary longitudinal analysis to a new α-synuclein transgenic mouse model. We show that early-stage decreases in dopamine release and vesicle reclustering precede late-stage changes in neuronal firing properties, measured by in vivo recordings from vulnerable neurons. Accumulated deficits in dopamine neurotransmission and altered neuronal firing are associated with cell death and motor abnormalities, in the absence of protein aggregation in the substantia nigra. These findings have important implications for developing therapies.

The LIM homeobox gene *Isl1* is required for the correct development of the striatonigral pathway in the mouse

Lisa A. Ehrman, Xiuqian Mu, Ronald R. Waclaw, Yutaka Yoshida, Charles V. Vorhees, William H. Klein, and Kenneth Campbell

The basal ganglia process cortical information that controls purposeful movements and appropriate behavior via the striatopallidal and striatonigral pathways. Despite their importance, little is known about the developmental mechanisms that control the formation of these pathways. We show here (pp. E4026–E4035) that telencephalic progenitors expressing the transcription factor Islet1 give rise to striatonigral neurons and that this factor is required for normal development of the striatonigral pathway. Moreover, Islet1 mouse mutants exhibit hyperactivity and a paradoxical response to psychostimulants. Given that the underlying causes of basal ganglia disorders such as attention deficit hyperactivity disorder (ADHD) are unknown, these findings may implicate possible alterations in neural circuitry.

Heme impairs the ball-and-chain inactivation of potassium channels

Nirakar Sahoo, Nishit Goradia, Oliver Ohlenschläger, Roland Schönherr, Manfred Friedrich, Winfried Plass, Reinhard Kappl, Toshinori Hoshi, and Stefan H. Heinemann

Heme, traditionally viewed as a stable protein cofactor such as in hemoglobin, also serves as an acute signaling molecule and is cytotoxic at high concentrations. Here (pp. E4036–E4044), we show that free intracellular heme potently enhances A-type potassium channel function. Such channels determine action potential frequency in excitable cells, and their dysfunction often contributes to pathological hyperexcitability, such as in pain and epilepsy. Binding of free heme at nanomolar concentrations to the "ball-and-chain" N terminus of A-type potassium channels, which typically closes the channels, introduces a stable structure in the otherwise disordered region and allows for a greater efflux of potassium ions, thus reducing cellular excitability. Heme therefore could be a powerful negative-feedback regulator in brain and muscle function.

Inter- and intrasubunit interactions between transmembrane helices in the open state of P2X receptor channels

Gabriel Heymann, Jian Dai, Mufeng Li, Shai D. Silberberg, Huan-Xiang Zhou, and Kenton J. Swartz

The opening of P2X receptor channels by extracellular ATP underlies purinergic signaling in many tissues. Here (pp. E4045–E4054) we use computational and functional approaches to study helix interactions within the transmembrane domain of P2X receptors. Our results suggest that the intersubunit crevices observed in the X-ray structure of detergent-solubilized ATP-bound receptors are nonnative but confirm helix interactions within individual subunits observed in both apo and ATP-bound receptors and identify a hot spot within a narrow internal region where the gating and permeation properties can be readily tuned.