PNAS Plus Significance Statements

ER stress stimulates production of the key antimicrobial peptide, cathelicidin, by forming a previously unidentified intracellular S1P signaling complex

Kyungho Park, Hiroko Ikushiro, Ho Seong Seo, Kyong-Oh Shin, Young il Kim, Jong Youl Kim, Yong-Moon Lee, Takato Yano, Walter M. Holleran, Peter Elias, and Yoshikazu Uchida

The cathelicidin antimicrobial peptide (CAMP) is an innate immune element that promotes antimicrobial defense, but excessive CAMP can stimulate inflammation and tumorigenesis. We recently discovered that external perturbations that induce subtoxic levels of endoplasmic reticulum (ER) stress increase sphingosine-1-phosphate (S1P) production, in turn activating NF-κB–mediated CAMP synthesis. We report here that S1P interacts with the heat shock proteins (HSP90α and GRP94) through a previously unidentified S1P receptor-independent intracellular mechanism, followed by the activation of NF-κB leading to stimulation of CAMP production. These studies illuminate the critical role of both ER stress and S1P in orchestrating stress-specific signals that enhance innate immunity. (See pp. E1334–E1342.)

Quantitative proteomic analyses of mammary organoids reveals distinct signatures after exposure to environmental chemicals

Katherine E. Williams, George A. Lemieux, Maria E. Hassis, Adam B. Olshen, Susan J. Fisher, and Zena Werb

We demonstrate that exposure to three environmental chemicals suggested to affect breast development—bisphenol A, mono-n-butyl phthalate, and polychlorinated biphenyl 153—at physiologically relevant doses results in unique responses and alterations in the proteome. This study provides insights into how the mammary epithelium changes in response to physiologically relevant exposures to xenobiotic chemicals. These changes could be correlated with increased risk of transformation or important changes in function. (See pp. E1343–E1351.)

Rare recombination events generate sequence diversity among balancer chromosomes in *Drosophila melanogaster*

Danny E. Miller, Kevin R. Cook, Nazanin Yeganeh Kazemi, Clarissa B. Smith, Alexandria J. Cockrell, R. Scott Hawley, and Casey M. Bergman

Balancer chromosomes are highly rearranged chromosomes that suppress recombination and are an important tool in *Drosophila* genetics, yet their precise molecular structure is unknown. Here we characterize the inversion breakpoints of the *X* chromosome balancer *FM7*, and provide evidence that rare double-crossover events with balanced homologs can occur. These rare exchange events do not undermine the use of balancers, but lead to diversity among balancers. We also provide genomic evidence that unequal exchange between duplicated regions underlies reversion at the *Bar* locus. Our work demonstrates the power of genome sequencing to understand the molecular nature of classical genetic resources, and cautions that mutations maintained over balancers in regions susceptible to exchange should be checked regularly to prevent their loss. (See pp. E1352–E1361.)

Differences in codon bias and GC content contribute to the balanced expression of TLR7 and TLR9

Zachary R. Newman, Janet M. Young, Nicholas T. Ingolia, and Gregory M. Barton

Codon bias, the unequal use of synonymous codons to encode amino acids, is known to influence protein expression. Our work finds that codon bias can limit the expression of Toll-like receptor 7, a receptor implicated in autoimmune diseases such as lupus. Surprisingly, we find that the improved protein production associated with codon optimization is not caused by increased translation but instead is caused by an increased rate of transcription. We attribute this change in transcription to increased guanine-cytosine (GC) content following codon optimization. Thus, our work not only addresses a fundamental aspect of immune regulation but also provides mechanistic insight into the controversial issue of how codon bias and GC content influence protein expression. (See pp. E1362–E1371.)

Emergence of functional subnetworks in layer 2/3 cortex induced by sequential spikes in vivo

Taekeun Kim, Won Chan Oh, Joon Ho Choi, and Hyung-Bae Kwon

Somatosensory information is transmitted and processed in the superficial layer (layer 2/3) of the cortex, giving rise to proper sensory perception. These processes ought to be dependent on the patterns of neuronal connectivity among layer 2/3 neurons, but underlying cellular mechanisms that govern how one neuron makes specific connections with other neurons and eventually builds functional microcircuits are not fully understood. We found that spikes generated in multiple neurons in vivo induced the formation of a functional group of neurons. Further data demonstrated that functional connectivity was determined by

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the order of the spike sequence and the number of neurons but not by the physical distance among neurons. These results imply that time-sensitive neuronal activity determines the pattern of circuit connectivity. (See pp. E1372–E1381.)

Presynaptic serotonin 2A receptors modulate thalamocortical plasticity and associative learning

Alexander Barre, Coralie Berthoux, Dimitri De Bundel, Emmanuel Valjent, Joël Bockaert, Philippe Marin, and Carine Bécamel

Higher-level cognitive processes strongly depend on a complex interplay between the mediodorsal thalamus and the prefrontal cortex. Alteration of thalamofrontal connectivity has been involved in schizophrenia. Prefrontal serotonin $(5\text{-HT})_{2A}$ receptors play an essential role in cortical network activity but the mechanism underlying their modulation of synaptic plasticity remains unexplored. Here, we demonstrate—to our knowledge for the first time—a physiological role of presynaptic 5-HT_{2A} receptors in the NMDA-operated induction of temporal-dependent plasticity at thalamocortical synapses. We show that $5\text{-HT}_{2A}^{-/-}$ mice exhibit alterations in plasticity and associative memory that are rescued by re-expressing receptors in the thalamus. These findings highlight a critical role of presynaptic 5-HT_{2A} receptor dysfunction in cognitive symptoms observed in schizophrenia. (See pp. E1382–E1391.)

Contrasting responses within a single neuron class enable sex-specific attraction in *Caenorhabditis elegans*

Anusha Narayan, Vivek Venkatachalam, Omer Durak, Douglas K. Reilly, Neelanjan Bose, Frank C. Schroeder, Aravinthan D. T. Samuel, Jagan Srinivasan, and Paul W. Sternberg

Roundworms carry out crucial sensory behaviors with a relatively small number of neurons. We find that male roundworms have strong preferences for particular concentrations of sex-specific small molecule cues secreted by their potential mates. These preferences emerge from the dynamics of a population of four apparently identical male-specific neurons. The response of these sensory neurons is not uniform, with some being excitatory and others inhibitory, and the timing of response varies with concentration. These features allow this single neuronal class to prefer a concentration, and potentially to calculate a derivative of chemical concentration. This previously uncharacterized neural coding strategy might allow nematodes to efficiently use a small number of cells to carry out a crucial computation to enact innate social behaviors. (See pp. E1392–E1401.)

Circadian misalignment increases cardiovascular disease risk factors in humans

Christopher J. Morris, Taylor E. Purvis, Kun Hu, and Frank A. J. L. Scheer

Shift work is a risk factor for hypertension, inflammation, and cardiovascular disease, even after controlling for traditional risk factors. Shift workers frequently undergo circadian misalignment (i.e., misalignment between the endogenous circadian system and 24-h environmental/behavioral cycles). This misalignment has been proposed to explain, in part, why shift work is a risk factor for hypertension, inflammation, and cardiovascular disease. However, the impact of circadian misalignment per se on 24-h blood pressure and inflammatory markers is poorly understood. We show—under highly controlled laboratory conditions—that short-term circadian misalignment increases 24-h blood pressure and inflammatory markers in healthy adults. Our findings may help explain why shift work increases hypertension, inflammation, and cardiovascular disease risk. (See pp. E1402–E1411.)