PNAS Plus Significance Statements

Early (300—100 B.C.) temple precinct in the Valley of Oaxaca, Mexico

Elsa M. Redmond and Charles S. Spencer

Recent excavations at the site of El Palenque have recovered the earliest-known temple precinct in the Valley of Oaxaca, Mexico, dating to the Late Monte Albán I phase (300–100 B.C.). This precinct exhibits characteristics similar to the temple precincts of 16th century Mesoamerican states. A walled enclosure contains differentiated temples, priests' residences, and ritual features. We propose (pp. E1707–E1715) that the precinct's components represent a hierarchy of temples staffed by a specialized full-time priesthood. A series of radiocarbon dates indicate that the El Palenque temple precinct was in use during the 300–100 B.C. period of archaic state emergence in Oaxaca.

Structures of complexes comprised of *Fischerella* transcription factor HetR with *Anabaena* DNA targets

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DNA palindromes were crystallized in complexes with HetR, a transcription factor required for heterocyst differentiation in the nitrogen-fixing cyanobacterium *Anabaena*. In three complexes, we observed (pp. E1716–E1723) hydrogen bonding of a single glutamate side chain with three successive cytosines in the DNA. The feature of three successive GC pairs in each arm of the palindrome is conserved in other filamentous cyanobacteria. These cyanobacteria contain HetR proteins, each of which contains glutamate in that critical position. This unique interaction between a protein factor and its DNA target is so important that it is invariant across cyanobacteria from environments around the world.

E4orf4 induces PP2A- and Src-dependent cell death in *Drosophila melanogaster* and at the same time inhibits classic apoptosis pathways

Antonina Pechkovsky, Maoz Lahav, Eliya Bitman, Adi Salzberg, and Tamar Kleinberger

Expression of the adenovirus protein E4orf4 alone in cultured mammalian cells prompts noncanonical apoptosis that is more efficient in oncogene-transformed cells than in normal cells. Here (pp. E1724–E1733), E4orf4 activity in a whole organism (*Drosophila melanogaster*) is described, leading to three significant conclusions: (*i*) E4orf4-induced cell death is an evolutionarily conserved process; (*ii*) E4orf4 induces a distinctive mode of cell death, differing from well-characterized cell death mechanisms; and (*iii*) E4orf4 activates cell death but concomitantly inhibits it, thus minimizing damage to normal tissues. The last finding suggests a possible explanation for the differential effect of E4orf4 in normal and cancer cells.

Castor is required for Hedgehog-dependent cell-fate specification and follicle stem cell maintenance in *Drosophila* oogenesis

Yu-Chiuan Chang, Anna C.-C. Jang, Cheng-Han Lin, and Denise J. Montell

Understanding the molecules that govern production of specific cell types from adult tissue stem cells is a major challenge, and *Drosophila* follicle stem cells (FSCs) are an outstanding model. We report (pp. E1734–E1742) identification of a gene, *castor*, which is required for FSC maintenance. Castor also functions in a genetic circuit with two other genes, *hedgehog* and *eyes absent*, to determine specific progeny cell fates. Our studies provide a marker for the earliest cell-fate decisions in this model and insight into the molecular and cellular mechanisms by which stem cells produce specific and diverse types of progeny.

Genomic rearrangements and the evolution of clusters of locally adaptive loci

Sam Yeaman

Genome scans often find that the loci involved in local adaptation tend to cluster together on chromosomes. A leading explanation suggests that clusters evolve because the probability of a new mutation establishing is higher when occurring near another locally adapted mutation, because such architectures are seldom disrupted by recombination. I show (pp. E1743–E1751) that this theory is unlikely to explain empirically observed clusters. Instead, simulations show that clusters are more likely to form through genomic rearrangements that bring coadapted loci close together. This suggests that ecological selection may play an important role in shaping genome architecture, in contrast to many nonadaptive explanations.

Dnr1 mutations cause neurodegeneration in *Drosophila* by activating the innate immune response in the brain

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Infection triggers the innate immune response in all metazoans, activating regulatory pathways that result in expression of effector proteins, including potent antimicrobial peptides. These pathways can also be activated in the brain by aging, stress, and injury. Although nominally protective, excessive neuroinflammatory responses may themselves contribute to neurodegenerative disease by mechanisms that remain unclear. We found (pp. E1752–E1760) that hyperactivation of innate immunity in the *Drosophila* brain as a result of mutation or bacterial injection causes neurodegeneration because of neurotoxic effects of antimicrobial peptides. These findings have important implications for the role of neuroinflammation in human neurodegenerative disease.

Soluble IL7Rα potentiates IL-7 bioactivity and promotes autoimmunity

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Many genes have been shown to influence the risk of developing multiple sclerosis (MS); however, the biological processes responsible are not clear. We found (pp. E1761–E1770) that a genetic polymorphism associated with increased MS risk is responsible for potentiating the effects of a cytokine named interleukin (IL)-7 by securing its availability and bioactivity over time. This effect was mediated by an isoform of the IL-7 receptor that circulates at high levels in blood. IL-7 is an important factor for T-cell maturation and proliferation, and, hence, its association to MS, which is an autoimmune disease, is not surprising.

The molecular basis for Mucosal-Associated Invariant T cell recognition of MR1 proteins

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Mucosal-associated invariant T (MAIT) cells are a highly conserved lineage of αβ T cells found in most mammals. These cells express a T-cell receptor of low diversity that recognizes vitamin metabolites presented by the MHC-related protein, MR1. Despite the evolutionary divergence of MR1 from other MHC proteins, we have found (pp. E1771–E1778) that MAIT T-cell receptors recognize MR1 using similar molecular strategies as that of the highly diverse, conventional αβ T cells, which recognize classical MHC molecules presenting peptide fragments. Our results also shed light onto how MR1-presented antigens can modulate the MAIT–T-cell receptor affinity and MAIT cell stimulation.

Aurora kinase inhibitors reveal mechanisms of HURP in nucleation of centrosomal and kinetochore microtubules

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In mitosis, microtubules extend and shrink before the bilateral attachment is established. However, which molecules regulate this activity for spindle formation is not fully elucidated. Using two in-house developed small molecules that target the Aurora kinases, we show that hepatoma up-regulated protein (HURP) is highly dynamic, trafficking between centrosome and kinetochore driven by Aurora A-dependent phosphorylation and protein phosphatase 1/2A-associated dephosphorylation. These compounds demonstrate a spatial hierarchical preference of HURP in the attachment of microtubules extending from the mother to the daughter centrosome. These findings (pp. E1779–E1787) help explain the biology of mitosis and may lead to the development of anticancer compounds.

Mutual antagonism between hypoxia-inducible factors 1α and 2α regulates oxygen sensing and cardio-respiratory homeostasis

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The carotid body (CB) chemosensory reflex and catecholamine secretion by the adrenal medulla (AM) are principal regulators of cardio-respiratory function during hypoxia, but the molecular mechanisms by which the CB and AM respond to hypoxia with changes in breathing and blood pressure are unknown. Hypoxia-inducible factor-1 (HIF-1) and HIF-2 mediate adaptive transcriptional responses to hypoxia. Herein, we demonstrate (pp. E1788–E1796) that a mutual functional antagonism between HIF-1 and HIF-2 plays a critical role in O_2 sensing by establishing a dynamic balance that determines the proper redox set point in the CB and AM, which is essential for maintenance of cardio-respiratory homeostasis.

Mapping remodeling of thalamocortical projections in the living *reeler* mouse brain by diffusion tractography

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Alterations of brain connectional circuits are often associated with developing brain disorders. Pathology, however, can also trigger adaptive brain plasticity and compensatory connectivity changes. This paper (pp. E1797–E1806) provides a verified noninvasive framework for high-resolution mapping of living mouse brain connectional anatomy. We show that pathological changes in the formation of the cortical sheet, such as gross laminar distortions induced by *reelin* gene mutation in mice, lead to spectacular compensatory remodeling of thalamocortical projections. Our findings reveal extensive brain plasticity in the *reeler* mutant mouse, a frequently used model of brain developmental pathology, with great translational value for human brain disorders.

ApoE influences amyloid-β (Aβ) clearance despite minimal apoE/Aβ association in physiological conditions

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It has been proposed that differential physical interactions of apolipoprotein E (apoE) isoforms with soluble amyloid-β (Aβ) in brain fluids influence the metabolism of Aβ, providing a major mechanism to account for how *APOE* influences Alzheimer's disease risk. The current study (pp. E1807–E1816) challenges this proposal and clearly shows that lipoproteins containing apoE isoforms are unlikely to play a significant role in Aβ metabolism by binding directly to Aβ in physiological fluids such as cerebrospinal fluid or interstitial fluid. Our in vitro and in vivo results suggest that apoE isoforms influence Aβ metabolism by competing for the same clearance pathways within the brain.

TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity

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This study (pp. E1817–E1826) shows that neurodegenerative changes induced by α-synuclein in midbrain dopamine neurons in vivo can be blocked through activation of the autophagy-lysosome pathway. Using an adeno-associated virus model of Parkinson disease to overexpress α-synuclein in the substantia nigra, we show that genetic [transcription factor EB (TFEB) and Beclin-1 overexpression] or pharmacological (rapalog) manipulations that enhance autophagy protect nigral neurons from α-synuclein toxicity, but inhibiting autophagy exacerbates α-synuclein toxicity. The results provide a mechanistic link between α-synuclein toxicity and impaired TFEB function, and identify TFEB as a target for therapies aimed at neuroprotection and disease modification in Parkinson disease.

Control of cell proliferation, endoreduplication, cell size, and cell death by the retinoblastoma-related pathway in maize endosperm

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Cereal endosperm is a key source of dietary calories and raw materials for countless manufactured goods. Understanding how the cell cycle is regulated during endosperm development could lead to increased crop yield. We show (pp. E1827–E1836) that a maize *Retinoblastoma*-related gene, *RBR1*, plays a central role in regulating gene expression, endoreduplication, and the number, size, and death of endosperm cells. *RBR1* is genetically coupled to *Cyclin Dependent Kinase A;1* in controlling endoreduplication but not gene expression. Seeds down-regulated for *RBR1* develop normally, which suggests higher-order control mechanisms regulating endosperm development that are superimposed on cell cycle regulation.