PNAS Plus Significance Statements

Absolute position total internal reflection microscopy with an optical tweezer

Lulu Liu, Alexander Woolf, Alejandro W. Rodriguez, and Federico Capasso

Total internal reflection microscopy is a low noise, minimally invasive, near surface particle-tracking technique widely used in physics and biology for the precise investigation of surface forces and interactions and fluorescent imaging of live cells and single molecules. In total internal reflection microscopy (TIRM), a particle is tracked, with nanometer precision, by the intensity of light it scatters from the evanescent field of a totally internally reflected beam of light. The present work (pp. E5609–E5615) introduces the first to our knowledge in situ, absolute position calibration method for TIRM (and total internal reflection fluorescence microscopy), which has the potential to greatly expand its measurement capabilities and bring quantitative results to studies using this technique.

Links that speak: The global language network and its association with global fame

Shahar Ronen, Bruno Gonçalves, Kevin Z. Hu, Alessandro Vespignani, Steven Pinker, and César A. Hidalgo

People have long debated about the global influence of languages. The speculations that fuel this debate, however, rely on measures of language importance—such as income and population—that lack external validation as measures of a language's global influence. Here we introduce a metric of a language's global influence based on its position in the network connecting languages that are co-spoken. We show (pp. E5616–E5622) that the connectivity of a language in this network, after controlling for the number of speakers of a language and their income, remains a strong predictor of a language's influence when validated against two independent measures of the cultural content produced by a language's speakers.

Electrically pumped semiconductor laser with monolithic control of circular polarization

Patrick Rauter, Jiao Lin, Patrice Genevet, Suraj P. Khanna, Mohammad Lachab, A. Giles Davies, Edmund H. Linfield, and Federico Capasso

As powerful semiconductor laser sources open up new possibilities for the realization of compact and versatile spectroscopy and detection systems, monolithic control of the laser output characteristics becomes essential. Whereas engineering of spectral characteristics and beam shape has reached a high level of maturity, manipulation of the polarization state remains challenging. We present (pp. E5623–E5632) a method for monolithic control of the degree of circular polarization by aperture antennas forming a surface-emitting grating on a semiconductor laser cavity and demonstrate its realization for a terahertz quantum cascade laser. Our approach is not limited to the terahertz regime and paves the way to an increased functionality and customizability of monolithic laser sources for a variety of applications (e.g., vibrational circular dichroism spectroscopy).

Proteogenomic analysis and global discovery of posttranslational modifications in prokaryotes

Ming-kun Yang, Yao-hua Yang, Zhuo Chen, Jia Zhang, Yan Lin, Yan Wang, Qian Xiong, Tao Li, Feng Ge, Donald A. Bryant, and Jin-dong Zhao

Proteogenomics is the application of mass spectrometry-derived proteomic data for testing and refining predicted genetic models. Cyanobacteria, the only prokaryotes capable of oxygenic photosynthesis, are the ancestor of chloroplasts in plants and play crucial roles in global carbon and nitrogen cycles. An integrated proteogenomic workflow was developed, and we tested this system on a model cyanobacterium, *Synechococcus* 7002, grown under various conditions. We obtained (pp. E5633–E5642) a nearly complete genome translational profile of this model organism. In addition, a holistic view of posttranslational modification (PTM) events is provided using the same dataset, and the results provide insights into photosynthesis. The entire proteogenomics pipeline is applicable to any sequenced prokaryotes and could be applied as a standard part of genome annotation projects.

Bifurcation analysis of single-cell gene expression data reveals epigenetic landscape

Eugenio Marco, Robert L. Karp, Guoji Guo, Paul Robson, Adam H. Hart, Lorenzo Trippa, and Guo-Cheng Yuan

Characterization of cellular heterogeneity and hierarchy are important tasks in developmental biology and may help overcome drug resistance in treatment of cancer and other diseases. Single-cell technologies provide a powerful tool for detecting rare cell types and cell-fate transition events, whereas traditional gene expression profiling methods can be used only to measure the average behavior of a cell population. However, the lack of suitable computational methods for single-cell data analysis has become a bottleneck. Here we present a method with the focuses on automatically detecting multilineage transitions and on modeling the associated changes in gene expression patterns. We show (pp. E5643–E5650) that our method is generally applicable and that its applications provide biological insights into developmental processes.

Hedgehog-induced phosphorylation by CK1 sustains the activity of Ci/Gli activator

Qing Shi, Shuang Li, Shuangxi Li, Alice Jiang, Yongbin Chen, and Jin Jiang

Hedgehog (Hh) signaling controls development and tissue homeostasis through the Cubitus interruptus (Ci)/glioma-associated oncogene homolog (Gli) transcription factors, and abnormal Gli activity causes congenital diseases and cancers. Here (pp. E5651–E5660) we show that Ci/Gli phosphorylation by Casein kinase 1 positively regulates Hh pathway activity, providing insights into the regulation of Ci/Gli activity. By showing that phosphorylation protects the Ci/Gli activator from premature degradation, our study not only sheds lights on how the production and degradation of Ci/Gli activator are delicately balanced to achieve optimal pathway activity but also provides the first evidence (to our knowledge) that protein degradation by the Cullin 3 family of E3 ubiquitin ligases is negatively regulated by phosphorylation.

Prehistoric genomes reveal the genetic foundation and cost of horse domestication

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The domestication of the horse revolutionized warfare, trade, and the exchange of people and ideas. This at least 5,500-y-long process, which ultimately transformed wild horses into the hundreds of breeds living today, is difficult to reconstruct from archeological data and modern genetics alone. We therefore sequenced two complete horse genomes, predating domestication by thousands of years, to characterize the genetic footprint of domestication. These ancient genomes (pp. E5661–E5669) reveal predomestic population structure and a significant fraction of genetic variation shared with the domestic breeds but absent from Przewalski's horses. We find positive selection on genes involved in various aspects of locomotion, physiology, and cognition. Finally, we show that modern horse genomes contain an excess of deleterious mutations, likely representing the genetic cost of domestication.

TORC1 regulators Iml1/GATOR1 and GATOR2 control meiotic entry and oocyte development in *Drosophila*

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The target of rapamycin complex 1 (TORC1) promotes cell growth and anabolic metabolism. In yeast, entry into meiosis is contingent on the down-regulation of TORC1 activity by the increased minichromosome loss 1/GTPase-activating proteins toward Rags 1 (Iml1/GATOR1) complex in response to amino acid starvation. Here (pp. E5670–E5677) we define the developmental requirements for the TORC1 regulators Iml1/GATOR1 and GATOR2 during *Drosophila* oogenesis. We demonstrate that, as is observed in yeast, the Iml1/GATOR1 complex down-regulates TORC1 activity to facilitate the mitotic/meiotic transition in *Drosophila* ovarian cysts. Later in oogenesis, components of the GATOR2 complex oppose the activity of GATOR1 to enable a rise in TORC1 activity that drives oocyte development and growth. Thus, a conserved nutrient stress pathway has been incorporated into a developmental program that regulates meiotic progression in *Drosophila*.

Essential role for autophagy during invariant NKT cell development

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Autophagy is an evolutionarily conserved catabolic process essential to maintaining cellular homeostasis through the breakdown and recycling of damaged organelles and long-lived proteins. We report (pp. E5678–E5687) that autophagy plays an essential cell-intrinsic role in maintaining the survival of a subset of innate-like cells known as invariant natural killer T (iNKT) cells. Autophagy deficiency prevents transition to a quiescent state after population expansion of thymic iNKT cells. Hence, autophagy-deficient iNKT cells accumulate mitochondria and oxygen radicals and subsequently die of apoptosis.

Licensed human natural killer cells aid dendritic cell maturation via TNFSF14/LIGHT

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As well as having potent cytotoxic activity, natural killer (NK) cells have a regulatory role and interactions between NK cells and dendritic cells (DCs) aid DC maturation and adaptive immunity. However, the mechanisms underpinning NK–DC cross-talk are poorly defined. We show (pp. E5688–E5696) that tumor cells induce rapid production of the cytokine TNF superfamily member 14 (TNFSF14) in human NK cells and that these NK cells induce DC maturation in a TNFSF14-dependent manner. The synergistic activity of NK cell activation receptors in licensed NK cells couples the release of cytotoxic granules to TNFSF14 production. Thus, NK cell activation by tumor cells is linked to the initiation of adaptive immunity via TNFSF14-mediated NK–DC cross-talk.

GDM-associated insulin deficiency hinders the dissociation of SERT from ERp44 and down-regulates placental 5-HT uptake

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Our findings (pp. E5697–E5705) provide insight on the molecular mechanism in which insulin regulates the dissociation of ERp44, an

endoplasmic reticulum chaperon, from the serotonin (5-HT) transporter (SERT) following the completion of disulfide bond formation. Furthermore, our data show that gestational diabetes mellitus-associated defects in insulin signaling tethers SERT with ERp44, at the intracellular compartment which down-regulates 5-HT uptake rates of the placental trophoblast. All the trophoblast used in these studies were isolated and purified directly from healthy or GDM placentas in our laboratories.

Estrogen-related receptor α is required for efficient human cytomegalovirus replication

Jesse Hwang, John G. Purdy, Kai Wu, Joshua D. Rabinowitz, and Thomas Shenk

Viruses use the host cell's resources, including numerous cellular metabolites, to successfully replicate and produce progeny. Here (pp. E5706–E5715), we report that estrogen-related receptor α (ERRα), an orphan nuclear receptor and transcriptional regulator, supports the expression of multiple metabolic enzymes that contribute to the metabolic program induced by human cytomegalovirus in cultured primary fibroblasts. Loss or inhibition of ERRα impedes the viral replication cycle at multiple levels, leading to a reduction in progeny virus. Our findings identify ERRα as a host factor that is manipulated by the virus for its replicative needs and establish its potential as a pharmacological target for treatment of cytomegalovirus infection.

STAT3 promotes survival of mutant photoreceptors in inherited photoreceptor degeneration models

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There is a great need for a therapy for inherited photoreceptor degenerations (IPDs) that would be effective irrespective of the affected gene. A treatment that slows photoreceptor (PR) death, even by 10–20%, could greatly extend the years of useful vision of patients with IPD for whom specific treatment is not available. Up-regulation of *Stat3* has been reported in the retinas of many IPD models, but whether *Stat3* expression is neuroprotective in mutant PRs has not been established. We show that *Stat3* plays a strong prosurvival role in two distinct IPD models and that further augmentation of PR *Stat3* expression slows PR death. These findings (pp. E5716–E5723) suggest the potential of *Stat3* augmentation, for example, by recombinant adenoassociated virus (rAAV) vector-mediated gene therapy, as a treatment for IPDs.