PNAS Plus Significance Statements

Compound facial expressions of emotion

Shichuan Du, Yong Tao, and Aleix M. Martinez

Though people regularly recognize many distinct emotions, for the most part, research studies have been limited to six basic categories—happiness, surprise, sadness, anger, fear, and disgust; the reason for this is grounded in the assumption that only these six categories are differentially represented by our cognitive and social systems. The results (pp. E1454–E1462) reported herein propound otherwise, suggesting that a larger number of categories is used by humans.

Molecular mechanism for differential recognition of membrane phosphatidylserine by the immune regulatory receptor Tim4

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Structural immunology employs static, atomic-level representations to uncover the molecular mechanisms by which immune receptors sensitively discern infection and disease. Lipid membranes, particularly those exposing phosphatidylserine lipids, are now understood to be important signals in various aspects of immune regulation. However, the dynamic nature of lipid membranes makes the standard tools of structural immunology poorly suited to these systems, leaving a large gap in our understanding. Here (pp. E1463–E1472) we implement a suite of multidisciplinary tools to demonstrate, for the first time to the authors' knowledge, a mechanism for sensitivity of an immune regulatory receptor (Tim4) to the membrane context of phosphatidylserine exposure. These findings uncover new aspects of Tim4's recognition properties and, perhaps more significantly, provide a methodology for obtaining atomic-level detail in membrane recognition.

From a structural average to the conformational ensemble of a DNA bulge

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Obtaining the conformational ensembles of biological macromolecules, beyond average structures, is extremely challenging but necessary for a complete understanding of their folding and functions. Such insights may also lead to the rational design of therapeutics that can target less-ordered macromolecules and may advance the design of nanostructures and nanomachines from nucleic acids. We have applied X-ray interferometry (pp. E1473–E1480) to estimate the conformational ensemble of a small-model macromolecule, a DNA bulge, representative of helix–junction–helix building blocks of natural RNAs and designed DNA nanostructures. The measured ensemble, in combination with molecular dynamics simulations, provides testable atomic-level models. X-ray interferometry is extremely sensitive and can detect changes in the ensemble arising from different bulge sequences and solution salt conditions.

Protein aggregation can inhibit clathrin-mediated endocytosis by chaperone competition

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The aggregation of mutant proteins is pathologically implicated in a large number of neuropathies, including Huntington disease and ALS. Although the appearance of protein aggregates is known to sequester other proteins, how this results in the gain-of-function toxicity in these diseases is unclear. Here (pp. E1481–E1490), we show that the aggregation of disease-associated proteins causes the reversible collapse of clathrin-mediated endocytosis (CME) and inhibits the internalization of membrane receptors that affect neuronal function. CME inhibition occurs through aggregate-mediated sequestration of the molecular chaperone heat shock cognate protein 70, which is essential for CME. We propose that a toxic "tug-of-war" occurs between aggregates and endogenous client proteins for available chaperones, leading to the collapse of multiple cellular processes in neurodegeneration and other protein conformation diseases.

Acentriolar mitosis activates a p53-dependent apoptosis pathway in the mouse embryo

Hisham Bazzi and Kathryn V. Anderson

Centrioles form the core of centrosomes, which organize cilia and interphase and spindle microtubules in animal cells, but centrosome function has not been defined in mammals in vivo. We show (pp. E1491–E1500) that mouse embryos that lack centrioles and centrosomes survive to midgestation, when they lack primary cilia and cilia-dependent signaling. Despite the absence of centrosomes, bipolar spindle formation, chromosome segregation, cell-cycle profile, and DNA damage response are normal in the mutants. Unlike mutants that lack cilia, most cells in acentriolar embryos activate a p53-dependent apoptotic pathway. The data show that mammalian centrioles promote the efficient and rapid assembly of the mitotic spindle and that a short delay in prometaphase activates a checkpoint that leads to p53-dependent cell death in vivo.

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Epidermal Wnt/β-catenin signaling regulates adipocyte differentiation via secretion of adipogenic factors

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The synchronized patterns of hair follicle growth and expansion of the dermal adipocyte layer have long been recognized. Although factors secreted by adipocytes are known to regulate the hair growth cycle, it is unclear whether, conversely, the epidermis can regulate adipogenesis. Our study (pp. E1501–E1509) now demonstrates that activation of epidermal Wnt/β-catenin signaling stimulates adipocyte differentiation in vivo and in vitro. The effect can be mediated by secreted factors, including insulin-like growth factor 2 and bone morphogenetic proteins 2 and 6.

Deepwater Horizon crude oil impacts the developing hearts of large predatory pelagic fish

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The 2010 *Deepwater Horizon* (MC252) disaster in the northern Gulf of Mexico released more than 4 million barrels of crude oil. Oil rose from the ocean floor to the surface where many large pelagic fish spawn. Here (pp. E1510–E1518) we describe the impacts of field-collected oil samples on the rapidly developing embryos of warm-water predators, including bluefin and yellowfin tunas and an amberjack. For each species, environmentally relevant MC252 oil exposures caused serious defects in heart development. Moreover, abnormalities in cardiac function were highly consistent, indicating a broadly conserved developmental crude oil cardiotoxicity. Losses of early life stages were therefore likely for Gulf populations of tunas, amberjack, swordfish, billfish, and other large predators that spawned in oiled surface habitats.

Overexpression of a DENND1A isoform produces a polycystic ovary syndrome theca phenotype

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Family-based studies revealed that polycystic ovary syndrome (PCOS), a common endocrinopathy of women, has a genetic basis. Genome-wide association studies identified *DENND1A* as a PCOS locus, but its role in PCOS was unknown. We report (pp. E1519–E1527) that an alternatively spliced form of *DENND1A* (DENND1A.V2) is increased in PCOS theca cells, the source of the excess androgens that characterizes PCOS. Forced expression of DENND1A.V2 in normal theca cells increased expression of genes encoding steroidogenic enzymes, leading to augmented androgen biosynthesis, whereas silencing of DENND1A.V2 in PCOS theca cells reverts them to a normal phenotype. Our findings establish that increased DENND1A.V2 expression is sufficient to promote a PCOS phenotype in human theca cells, information that can inform development of diagnostic tests as well as novel therapeutic interventions.

Heat shock protein 90 controls HIV-1 reactivation from latency

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Antiretroviral therapy cannot eradicate HIV-1 because the virus can become transcriptionally inactive in resting memory CD4+ T cells (and other cell types), which are long-lived, thus generating a reservoir undetectable by the immune system. When therapy is stopped, the latent viral reservoir is activated and HIV-1 rebounds. Our understanding of HIV-1 latency and reactivation is incomplete. Here (pp. E1528–E1537) we report that the heat shock protein 90 (Hsp90) regulates HIV-1 reactivation from latency by controlling the NF-kB pathway. Therefore Hsp90 is a key molecule linking HIV-1 reactivation from latency to CD4+ T-cell activation. Selective Hsp90 inhibitors combined with PKC-ϑ inhibitors, all in phase II clinical trials, potently suppressed HIV-1 reactivation, thus Hsp90 may be a novel target to control HIV-1 latency.

Uncoupling reproduction from metabolism extends chronological lifespan in yeast

Saisubramanian Nagarajan, Arthur L. Kruckeberg, Karen H. Schmidt, Evgueny Kroll, Morgan Hamilton, Kate McInnerney, Ryan Summers, Timothy Taylor, and Frank Rosenzweig

All cells age and do so in relation to how many times a cell divides (replicative aging) and how long a nondividing cell can live (chronological aging). Bakers' yeast has been used to study both, but because yeast divides when nutrient levels permit, the genetics of its chronological lifespan has only been studied under calorie restriction, mimicked by starvation. Because many terminally differentiated animal cells are long-lived and rarely starve, we developed a model of cell lifespan (pp. E1538–E1547) under calorie-unrestricted conditions. When encapsulated and fed ad libitum, yeast goes into cell cycle arrest, continues to be metabolically active, and remains viable for weeks, offering a new experimental paradigm to study chronological lifespan in the absence of calorie restriction.

Proteomic analysis of *Vibrio cholerae* outer membrane vesicles

Emrah Altindis, Yang Fu, and John J. Mekalanos

Identifying proteins localized on the surface and envelope of Gram-negative bacterial cells is an important problem in vaccine development and antibiotic target discovery. We show (pp. E1548–E1556) that the characterization of proteins associated with outer membrane vesicles (OMVs) released by Gram-negative cells provides a solution in that contamination with abundant cytoplasmic proteins (caused by cell lysis) can be avoided. Integrated at a systems level with other transcription and proteomic data sets, our research provides a view of the surface architecture of a pathogen undergoing host-programmed changes in gene expression. Also provided is the first evidence to our knowledge that secreted protein-folding quality control (a property of the DegP protease) influences the composition of OMVs and bacterial virulence, validating DegP as a target for virulence-blocking drugs.