PNAS Plus Significance Statements

Centrosomes are autocatalytic droplets of pericentriolar material organized by centrioles

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How cells position their proteins is still an open question. Here, we propose a physical description of centrosomes, which are membraneless organelles involved in cell division. In our model (pp. E2636–E2645), centrosome material occurs in a soluble form and a form that tends to form droplets by phase separation. We find that an autocatalytic chemical transition between these forms quantitatively accounts for our experimental data. Importantly, a catalytic activity of the centrioles, which are located inside centrosomes, can control centrosome nucleation and suppress Ostwald ripening to allow for two equal-sized centrosomes to coexist in the cell. Consequently, our example shows how the combination of chemical reactions and phase separation can be used to control the formation of liquid-like compartments in cells.

RNF144A, an E3 ubiquitin ligase for DNA-PKcs, promotes apoptosis during DNA damage

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In response to DNA damage, a proper balance between DNA repair and apoptosis is very important for genomic integrity. DNA-dependent protein kinase, catalytic subunit (DNA-PKcs) plays a key role in DNA repair. Many reports also indicate a prosurvival function for cytosolic DNA-PKcs. However, how this prosurvival activity of DNA-PKcs is regulated remains unknown. We identify (pp. E2646–E2655) Ring Finger protein 144A (RNF144A) as the first E3 ubiquitin ligase for cytosolic DNA-PKcs. RNF144A is induced in a p53-dependent manner during DNA damage and targets cytosolic DNA-PKcs for ubiquitination and degradation. The regulation of DNA-PKcs by RNF144A is important for proper apoptotic response during DNA damage, suggesting a tumor suppressor function for RNF144A.

Origins of specificity and affinity in antibody–protein interactions

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Natural antibodies perform their biological function by recognizing all sorts of foreign proteins—seemly unlimited structural and sequence diversities in antigens can be recognized by a limited repertoire of antibodies, for which the sequence and structure are relatively homogeneous. We found (pp. E2656–E2665) that the energetically critical epitope portions are largely composed of backbone atoms, side-chain carbons, and hydrogen bond donors/acceptors. These key components are ubiquitous on protein surfaces and can be recognized by the enriched aromatic side chains and, to a lesser extent, short-chain hydrophilic residues on the antibody paratopes; antibodies, with relatively limited sequence and structural diversities in the antigen binding sites, can recognize unlimited protein antigens through recognizing the common physicochemical features on all protein surfaces.

Single-residue insertion switches the quaternary structure and exciton states of cryptophyte light-harvesting proteins

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There is intense interest in determining whether coherent quantum processes play a nontrivial role in biology. This interest was sparked by the discovery of long-lived oscillations in 2D electronic spectra of photosynthetic proteins, including the phycobiliproteins (PBPs) from cryptophyte algae. Using X-ray crystallography (pp. E2666–E2675), we show that cryptophyte PBPs adopt one of two quaternary structures, open or closed. The key feature of the closed form is the juxtaposition of two central chromophores resulting in excitonic coupling. The switch between forms is ascribed to the insertion of a single amino acid in the open-form proteins. Thus, PBP quaternary structure controls excitonic coupling and the mechanism of light harvesting. Comparing organisms with these two distinct proteins will reveal the role of quantum coherence in photosynthesis.

MicroRNA binding to the HIV-1 Gag protein inhibits Gag assembly and virus production

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MicroRNAs normally function to regulate gene expression through RNA-interference–mediated gene silencing. Here, we demonstrate that microRNAs can inhibit HIV-1 virus production by a novel mechanism not involving RNAi-mediated interference. The new mechanism involves interactions between microRNA and HIV-1 Gag protein's RNA-binding (nucleocapsid) domain. These interactions prevent Gag proteins from effectively multimerizing into viral complexes at the plasma membrane and lead to inhibition of viral particle production. The microRNA–Gag interactions further result in Gag proteins being redirected into the endocytic pathway where they are degraded in lysosomes. These findings (pp. E2676–E2683) have significant implications for understanding how cells modulate HIV-1 infection and raise the possibility of manipulating total expression levels of host miRNAs to combat HIV-1 replication.

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Engineering ePTEN, an enhanced PTEN with increased tumor suppressor activities

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A major tumor suppressor, phosphatase and tensin homolog (PTEN), dephosphorylates the potent tumorigenic signaling lipid phosphatidylinositol (3,4,5)-trisphosphate (PIP3) at the plasma membrane. However, most PTEN is located in the cytosol and only transiently associated with the membrane to convert PIP3 to PIP2. Here (pp. E2684–E2693), we developed a platform using a heterologous expression system, in which a library of randomly mutated human PTEN is expressed and localization of the protein is visually inspected in *Dictyostelium*. This unbiased approach revealed a membrane-binding regulatory interface that is negatively regulated by a phosphorylated C-terminal tail. Based on the mechanistic information, we created an enhanced PTEN that dramatically represses PIP3 signaling. Thus, PTEN activation readjusts PIP3 signaling in tumor cells and serves as a target for anticancer therapies.

Time-varying, serotype-specific force of infection of dengue virus

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Using mathematical models to extend knowledge of pathogen transmission and recommend optimized control efforts is dependent on the accuracy of model parameters. The rate at which susceptible individuals become infected [the force of infection (FoI)] is one of the most important parameters, but due to data constraints it is often incorrectly assumed to be constant over time. Using a bespoke method for a 12-y longitudinal dataset of serotype-specific dengue virus (DENV) infections, we estimated time-varying, serotype-specific FoIs for all four DENV serotypes. The FoI varied markedly in time, which implies that DENV transmission dynamics are complex and are best summarized using time-dependent transmission parameters. Our results (pp. E2694–E2702) provide more accurate measures of virus transmission dynamics and a basis for improving selection of control and disease prevention strategies.

Dietary history contributes to enterotype-like clustering and functional metagenomic content in the intestinal microbiome of wild mice

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Recent investigation of several mammalian hosts suggests that their intestinal bacterial communities display evidence of clusters characterized by differences in specific bacterial taxa, a concept referred to as enterotypes. By performing stable isotope analysis of environmental samples, monitoring communities during dietary shifts, and collecting functional metagenomic sequence data, we provide novel insight into the origins and dynamics of enterotype-like community clustering in wild house mice (pp. E2703–E2710). Two clusters are present in wild mice, one associated with higher plant-derived and another with animal-derived food intake, which can shift within a period of 1 wk. Remarkably, these clusters display shared characteristics with those present in humans, chimpanzees, and laboratory mice, suggesting ancient shared traits among mammalian bacterial communities.

Normal levels of the antiprion proteins Btn2 and Cur1 cure most newly formed [URE3] prion variants

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The [URE3] prion (infectious protein) is an amyloid (filamentous polymer) of Ure2p. Overproduction of Btn2p or Cur1p cure this prion, and Btn2p colocalizes with Ure2p aggregates in the curing process. We show (pp. E2711–E2720) that most [URE3] variants arising in the absence of Btn2p and Cur1p are cured by restoring the normal levels of these two proteins. The variants cured by normal levels of Btn2p and Cur1p are those with low seed number, a low number of heritable particles, consistent with seed aggregation, and sequestration as the curing mechanism. These Btn2p and Cur1p antiprion proteins are members of the Hook protein family, metazoan members of which have roles in microtubule-dependent movement of organelles and aggregates in the cell.

BET and HDAC inhibitors induce similar genes and biological effects and synergize to kill in Myc-induced murine lymphoma

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Bromodomain and extraterminal (BET) proteins bind acetylated proteins, including histones, and regulate transcription. Interestingly, inhibitors of BET proteins (BETi) can block cancer cell proliferation and induce apoptosis in a wide range of tumor types. To date many of the effects of BETi have been attributed to transcriptional suppression of genes like the MYC oncogene. We show that genetically-engineered Myc-induced lymphoma mouse models are highly sensitive to BETi without MYC transcription being suppressed. Our data (pp. E2721–E2730) suggest broad effects on transcription by BETi including a set of genes being induced. Here a genetic and functional link between BET proteins and histone deacetylases is unraveled that opens up avenues for combination therapies against cancer.

Inhibition of Ninjurin 1 restores erectile function through dual angiogenic and neurotrophic effects in the diabetic mouse

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Curative treatment modalities for erectile dysfunction (ED) are not available. Penile erection is a neurovascular phenomenon, and ED is caused mainly by vascular and neurologic disturbances. Here (pp. E2731–E2740) we demonstrate that inhibition of nerve injury-induced protein 1 promotes penile angiogenesis and neural regeneration through angiopoietin-1–Tie2 signaling and rescues erectile function in diabetic mice. Our preclinical work shed light on the application of therapeutic angiogenesis and neural regeneration for the treatment of human ED.

Postsynaptic activity reverses the sign of the acetylcholine-induced long-term plasticity of $GABA_A$ inhibition

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Cholinergic activity regulates excitability and plasticity in neuronal circuits through the activation of muscarinic and nicotinic receptors. Here we demonstrate that muscarinic receptors can depress or enhance synaptic inhibition in the hippocampal CA1 region, depending on the quiescent or active state of the postsynaptic target CA1 pyramidal neuron, the main hippocampal CA1 output (pp. E2741–E2750). These effects regulate inhibition from a presynaptic to a postsynaptic site, a relocation that could be essential to control activity associated with cognitive functions and the homeostatic regulation of abnormal hyperexcitability.

Dopamine transporters govern diurnal variation in extracellular dopamine tone

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The mechanism for diurnal (i.e., light/dark) oscillations in extracellular dopamine tone in mesolimbic and nigrostriatal systems is unknown. This is because, unlike other neurotransmitter systems, variation in dopamine tone does not correlate with variation in dopamine cell firing. The current research pinpoints the dopamine transporter as a critical governor of diurnal variation in both extracellular dopamine tone and the intracellular availability of releasable dopamine. These data (pp. E2751–E2759) describe shifts in the function of the dopamine system over time, which may have implications for diurnal effects on dopamine-dependent learning, sleep/wake behavior, locomotor activity, reward, and drug addiction.

SHORT VEGETATIVE PHASE reduces gibberellin biosynthesis at the *Arabidopsis* shoot apex to regulate the floral transition

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In plants the transition from vegetative growth to flowering is induced by environmental cues. The amplitude of these responses is enhanced by repressors that strongly delay flowering under non-inductive conditions. In *Arabidopsis thaliana*, the transcription factor SHORT VEGETATIVE PHASE (SVP) has a major role among these repressors. We show that SVP has an unrecognized function in repressing biosynthesis of the plant growth regulator gibberellin (GA) at the shoot apex. Under inductive photoperiods, *SVP* expression falls, contributing to increased expression of a GA biosynthetic enzyme that accelerates flowering. These results (pp. E2760–E2769) link GA biosynthesis to the established regulatory network controlling flowering and illustrate one of the mechanisms by which the levels of growth regulators are synchronized with the floral transition.

Automated identification of stratifying signatures in cellular subpopulations

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Single-cell measurement technologies such as flow cytometry permit the investigation of specific cellular subpopulations. Mass cytometry currently measures >40 parameters per cell and produces phenotypically rich datasets that may be retrospectively interrogated for relevant biological signal. There are few methods that identify experimentally relevant subpopulations within these datasets, and most do not scale well to higher-dimensional measurements. To address this bottleneck, we present a data-driven method termed Citrus that identifies cell subsets associated with an experimental endpoint of interest (pp. E2770–E2777). Citrus can test diverse experimental hypotheses and is demonstrated through the systematic identification of (*i*) blood cells that signal in response to experimental stimuli and (*ii*) T-cell subsets whose abundance is predictive of AIDS-free survival risk in patients with HIV.