PNAS Plus Significance Statements

Highly sensitive detection of nanoparticles with a self-referenced and self-heterodyned whispering-gallery Raman microlaser

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To date, loss compensation in optical microresonators has been done using rare-earth ions, which requires additional processing steps and costs and raises biocompatibility concerns. An alternative to integrating rare-earth ions for loss compensation is the use of intrinsic gain mechanisms such as Raman and parametric gain present in the materials from which resonators are fabricated. Here (pp. E3836–E3844), we report the first implementation to our knowledge of Raman gain-induced loss compensation in silica whispering-gallery-mode (WGM) resonators for improved detection and the first demonstration to our knowledge of mode splitting in a WGM Raman microlaser for detecting and counting single nanoparticles down to 10 nm. This intrinsically self-referenced, self-heterodyned, and biocompatible scheme has enabled achieving record-high polarizability sensitivity (down to 3.82×10^{-6} μm^3) without using plasmonic effects, passive or active stabilization, or frequency locking.

Structural insight into the molecular mechanism of allosteric activation of human cystathionine β-synthase by *S*-adenosylmethionine

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Cystathionine β-synthase (CBS), the pivotal enzyme of the transsulfuration pathway, regulates flux through the pathway to yield compounds, such as cysteine, glutathione, taurine, and H_2S, that control cellular redox status and signaling. Our crystal structure of an engineered human CBS construct bound to *S*-adenosylmethionine (AdoMet) reveals the unique binding site of the allosteric activator and the architecture of the human CBS enzyme in its activated conformation. Together with the basal conformation that we reported earlier (pp. E3845–E3852), these structures unravel the molecular mechanism of human CBS activation by AdoMet. Current knowledge will allow for modeling of numerous pathogenic mutations causing inherited homocystinuria and for design of compounds modulating CBS activity.

Remodeling of a delivery complex allows ClpS-mediated degradation of N-degron substrates

Izarys Rivera-Rivera, Giselle Román-Hernández, Robert T. Sauer, and Tania A. Baker

Adaptor proteins often regulate substrate selection by AAA+ enzymes, but the molecular mechanisms of adaptor-mediated substrate delivery are poorly understood. We find (pp. E3853–E3859) that an unstructured N-terminal extension (NTE) of ClpS, the adaptor that delivers N-degron substrates to the ClpAP protease, enters the ClpA translocation pore during substrate delivery and must be actively engaged for delivery to occur. ClpA engagement of the ClpS NTE promotes delivery of substrate bound to the same adaptor molecule. These results support a model in which ClpA remodels ClpS by translocating its NTE, triggering delivery of the N-degron substrate. Active remodeling of components in delivery complexes by AAA+ unfoldases and proteases is likely a widespread mechanism.

Utilization of extracellular information before ligand-receptor binding reaches equilibrium expands and shifts the input dynamic range

Alejandra C. Ventura, Alan Bush, Gustavo Vasen, Matías A. Goldín, Brianne Burkinshaw, Nirveek Bhattacharjee, Albert Folch, Roger Brent, Ariel Chernomoretz, and Alejandro Colman-Lerner

Many cell decisions depend on precise measurements of external ligands reversibly bound to receptors. Yeast cells orient in gradients of sex pheromone detecting differences in the amount of ligand-receptor complex. However, yeast can orient in gradients with nearly all receptors occupied. We describe a general systems-level mechanism, pre-equilibrium sensing and signaling (PRESS), which overcomes this saturation limit by shifting and expanding the input dynamic range to which cells can respond. PRESS requires that events downstream of the receptor be transient and faster than the time required for the receptor to reach equilibrium binding. Experiments and simulations show that PRESS operates in yeast and may help cells orient in gradients (pp. E3860–E3869). Many ligand-receptor interactions are slow, suggesting that PRESS is widespread throughout eukaryotes.

TRPV6 calcium channel translocates to the plasma membrane via Orai1-mediated mechanism and controls cancer cell survival

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Transient receptor potential vanilloid subfamily member 6 (TRPV6) is a highly selective Ca^{2+} channel that exercises its normal physiological function via Ca^{2+} absorption in the intestine and kidney. Intriguingly, we show that the TRPV6 channel may switch from its well-known constitutive activity to the store operated due to the remodeling mechanism involving STIM1/Orai1/TRPC1-induced activation of TRPV6 channel translocation to the plasma membrane via the Ca^{2+}/Annexin I/S100A11 pathway. Moreover, we demonstrate (pp. E3870–E3879) that the discovered mechanism is used by prostate cancer cells. This channel is absent in healthy prostate and is expressed de novo in prostate cancer cells, where it changes the role by supplying Ca^{2+}, which is used in cancer to increase cell survival.

Controlled sumoylation of the mevalonate pathway enzyme HMGS-1 regulates metabolism during aging

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The mevalonate pathway plays a critical role in cholesterol homeostasis and cancer development, two major challenges in modern medicine. Consequently, cholesterol-reducing medications (statins) that target this pathway

are the best-selling pharmaceutical drugs in history. Beyond regulation of the enzyme HMG-CoA reductase, little is known about additional post-translational regulatory nodes in the mevalonate pathway or how this cascade is controlled with age. We have discovered (pp. E3880–E3889) a regulatory circuit that controls HMGS-1, the first enzyme of the mevalonate pathway, during aging. HMGS-1 is regulated by posttranslational ubiquitination and age-dependent sumoylation. Sumoylation is reversed by the spatiotemporally controlled activity of a specific small ubiquitin-like modifier protease. This conserved molecular circuit could serve as a handle for targeting the mevalonate pathway in future therapeutics.

Nup98 promotes antiviral gene expression to restrict RNA viral infection in *Drosophila*

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The innate immune system is a highly conserved mode of defense that induces gene expression programs to restrict microbial infections. However, much remains unknown about how the target genes are poised for their rapid induction. Using a *Drosophila* model, we demonstrate that Nup98 plays an essential antiviral role in insects against human insect-borne viruses. Although Nup98 is known for its role in nuclear-cytoplasmic transport, our data suggest that this antiviral function is not at the nuclear pore, rather at promoters controlling expression of a subset of virus-induced genes. Our findings (pp. E3890–E3899) suggest that the Nup98 primes virus-stimulated genes by regulating the occupancy of active RNA polymerase at these promoters poising them for rapid induction, thereby coordinating a robust and complex antiviral response.

One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities

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The 2003 severe acute respiratory syndrome (SARS) epidemic and recent emergence of Middle East respiratory syndrome highlight the potential lethality of zoonotic coronavirus infections in humans. No specific antiviral treatment options are available. Coronaviruses possess the largest known RNA virus genomes and encode a complex replication machinery consisting of 16 viral nonstructural proteins (nsps). Our study (pp. E3900–E3909) reveals that the SARS-coronavirus RNA polymerase (nsp12) needs to associate with nsp7 and nsp8 to activate its capability to replicate long RNA. Moreover, this complex associates with nsp14, the proofreading subunit required to safeguard coronavirus replication fidelity. Our study thus defines the core of an RNA-synthesizing machinery that is unique in the RNA virus world and includes several key targets for antiviral drug development.

Xylella fastidiosa outer membrane vesicles modulate plant colonization by blocking attachment to surfaces

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Release of outer membrane vesicles (OMVs) is a general feature of Gram-negative bacteria. Most studies have addressed the mechanisms of their formation or the cargo they can carry, but other roles remain to be explored further. Here (pp. E3910–E3918) we provide evidence for a novel role for OMVs in *Xylella fastidiosa*, a bacterial pathogen that colonizes the xylem of important crop plants. OMVs, whose production is suppressed by a quorum-sensing system, serve as an autoinhibitor of cell adhesion to surfaces, thereby blocking attachment-driven biofilm formation that would restrict movement within the xylem and thus colonization of plants. The ubiquity of OMV formation in the bacterial world suggests that these extracellular products may have alternative roles that might modulate movement and biofilm formation.

Connexin hemichannels contribute to spontaneous electrical activity in the human fetal cortex

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Young neurons require occasional bursts of action-potential firing to maintain intracellular processes, to drive gene expression, to indicate their presence in a new location, and to attract and keep synaptic contacts. While in the adult cortex electrical activity is driven by synaptic inputs, during early cortical development these synaptic inputs are largely absent. In the absence of synaptic connections and sensory experience, human neurons use an energetically favorable membrane mechanism for generating and maintaining electrical activity: connexin hemichannels (pp. E3919–E3928). The spontaneous flickering of connexin hemichannels produces depolarizing events (often crowned with bursts of action potentials) to help establish early electrical communication in young subplate neurons. This type of activity dominates the human cortical wall 5 months before birth.

C-terminal domain small phosphatase 1 and MAP kinase reciprocally control REST stability and neuronal differentiation

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A fundamental process involved in nervous-system formation is the conversion of stem cells into mature neurons. A key transcription factor in this regard is repressor element 1 (RE1) silencing transcription factor (REST), which suppresses the neuronal phenotype in stem cells and must be eliminated to promote the expression of neuronal genes in postmitotic neurons. We find (pp. E3929–E3936) that a phosphatase, C-terminal domain small phosphatase 1, coexpressed with REST in stem cells, dephosphorylates a newly identified site on REST and promotes REST stability. Conversely, we find that epidermal growth factor, an extracellular signaling molecule that promotes neurogenesis, induces phosphorylation by extracellular signal-regulated (ERK/MAP) kinases at the same site on REST. The phosphorylation facilitates elimination of REST during the transition to neurons. Our mechanism helps explain the timing of REST degradation during neuronal differentiation.

Mixtures of opposing phosphorylations within hexamers precisely time feedback in the cyanobacterial circadian clock

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Many organisms possess biological clocks that schedule their behavior throughout the day. To function properly, these clocks must maintain a period near 24 hours despite fluctuations in conditions. In a simple three-protein oscillator from cyanobacteria, timing information is stored in KaiC, a phosphorylated protein which forms hexameric rings. We show (pp. E3937–E3945) that the feedback loop that allows oscillation depends cooperatively on phosphorylation throughout the KaiC hexamer. Two phosphorylation sites with different kinetics have opposing effects, and this creates a sharp transition between the day and night states of the ring. This mechanism, based on opposing modifications, generates circadian rhythms across the relevant range of protein stochiometries and may be used generally in biochemical networks for precise timing.