PNAS Plus Significance Statements

Redox-dependent stability, protonation, and reactivity of cysteine-bound heme proteins

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Metal thiolates are common components of electron transfer proteins, enzymes, and redox sensors. Reduction of ferric thiolate hemes often involves protonation of the Cys ligand and ligand loss or substitution. These reactions contribute to enzyme inactivation and form the basis of sensing functions, but their mechanistic details are not well understood, particularly in proteins. A neutral thiol is a viable ligand to the heme; however, analysis of this interaction has been challenging. Herein (pp. E306–E315), we describe a series of model proteins that offer detailed spectroscopic and thermodynamic characterization of ferric thiolate and ferrous thiol species as well as their interconversions. Results have shed light on the forces that control a particular heme ligation and redox reactivity of these key bioinorganic centers.

Mismatch repair protein hMSH2–hMSH6 recognizes mismatches and forms sliding clamps within a D-loop recombination intermediate

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Recombination between divergent DNA sequences (homeologous recombination) is generally suppressed to preserve cellular genetic integrity and to ultimately introduce genetic barriers between species. Decades of genetic and cell biology studies have identified the involvement of the mismatch repair (MMR) machinery in the quality control of homologous recombination. However, the molecular mechanism by which this remarkable control is achieved is unknown. Here (pp. E316–E325), we report the biophysical reconstitution and analysis of the early steps in the rejection of divergent DNA sequences by the MMR machinery during recombination initiation. We have determined that the first responder of MMR, human MutS-homolog hMSH2–hMSH6, efficiently recognizes mismatches within a D-loop recombination initiation intermediate, even in the presence of recombination initiation proteins HsRAD51 and human replication protein A (HsRPA).

Information transfer by leaky, heterogeneous, protein kinase signaling systems

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Extracellular concentrations convey information to cells about their environment. To sense these signals, cells use biomolecular networks that exhibit inevitable cell-to-cell variability and basal activity. Basal activity is widespread under physiological conditions (with phenotypic consequences), is often raised in disease, and can eradicate the transfer of information. In an experimental study of ERK signaling by single cells exhibiting heterogeneous ERK expression and basal activity, we verify our central theoretical prediction: Negative feedback substantially increases information transfer to the nucleus by preventing a near-flat average response curve and reducing sensitivity to variation in the ERK expression level. Our results (pp. E326–E333) reveal an important role for negative feedback mechanisms in protecting information transfer by saturable cell signaling systems from basal activity.

Delineating cooperative responses of processive motors in living cells

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Although many vesicles and organelles are known to be transported by groups of interacting cytoskeletal motors, the precise impact of collective motor behaviors on intracellular transport and trafficking processes remains controversial. By engineering COS-7 cells (pp. E334–E343) to provide genetic control over the density of motors on, and the sizes of vesicles (peroxisomes), we performed systematic comparisons of the collective behaviors of kinesin and myosinVa motors. The responses of cargo velocities, run lengths, and position fluctuations to these parameters suggest that myosinVa motors can cooperate more productively than kinesins when transporting cargos as a team. This behavior is derived from the mechanochemical properties of these motors and suggests that the collective functions of motors like myosinV can be regulated more sensitively than those of kinesin.

Analysis of chromatin-state plasticity identifies cell-type–specific regulators of H3K27me3 patterns

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We developed (pp. E344–E353) a computational approach to characterize chromatin-state plasticity across cell types, using the repressive mark H3K27me3 as an example. The high plasticity regions (HPRs) can be divided into two functionally and mechanistically distinct groups, corresponding to CpG island proximal and distal regions, respectively. We identified cell-type–specific regulators correlating with H3K27me3 patterns at distal HPRs in ENCODE cell lines as well as in primary human erythroid precursors. We predicted and validated a previously unrecognized role of T-cell acute lymphocytic leukemia-1 (TAL1) in modulating H3K27me3 patterns through interaction with additional cofactors, such as growth factor independent 1B (GFI1B). Our integrative approach provides mechanistic insights into chromatin-state plasticity and is broadly applicable to other epigenetic marks.

Conserved TCP domain of Sas-4/CPAP is essential for pericentriolar material tethering during centrosome biogenesis

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In centrosomes, pericentriolar material (PCM) serves as the principle site for microtubule nucleation and anchoring. In *Drosophila*, the centrosomal protein spindle assembly defective-4 (Sas-4) scaffolds cytoplasmic PCM protein complexes via its N terminus and tethers them to centrioles via an unknown mechanism. By determining the crystal structure of Sas-4's C-terminal T complex protein 10 (TCP) domain and functional studies in *Drosophila*, human cells, and induced pluripotent stem cell-derived neural progenitors, we show that Sas-4 performs its tethering role via its TCP domain. Furthermore, point mutations within the TCP domain perturb PCM tethering while still allowing the protein to scaffold cytoplasmic PCM complexes. These studies (pp. E354–E363) provide insights into how Sas-4 proteins tether PCM complexes for the assembly of functional centrosomes.

Network of mutually repressive metastasis regulators can promote cell heterogeneity and metastatic transitions

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Cancer progression, as an evolutionary process, should accelerate if higher cellular variability is present. However, the sources of nongenetic variability in metastatic progression are largely unknown. To address this question, we characterized a transcriptional regulatory network for the metastasis suppressor Raf kinase inhibitory protein (RKIP). We previously showed that the transcription factor BACH1 is negatively regulated by RKIP and promotes breast cancer metastasis. Here (pp. E364–E373) we reveal a network architecture between the metastasis suppressor RKIP and the metastasis promoter BACH1 that enables single cells to generate a stable subpopulation of prometastatic cells without any genetic changes.

TGF-β directs trafficking of the epithelial sodium channel ENaC which has implications for ion and fluid transport in acute lung injury

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The acute respiratory distress syndrome (ARDS) is a devasting clinical problem with high mortality, no drug therapy, and poorly understood pathogenesis. The hallmark of ARDS is persistent pulmonary edema, attributable in part to impaired Na^+ and fluid transport across the alveolo-capillary barrier, undertaken by the epithelial sodium channel (ENaC). We describe (pp. E374–E383) a unique signaling pathway driven by TGF-β, which acutely dysregulates ENaC trafficking, blocking alveolar Na^+ transport and edema resolution. This pathway represents a unique pathomechanism in ARDS, highlights potential "druggable" targets, and may represent a physiological means of acutely regulating ENaC in lungs and other organs.

Hypoxia-inducible factors mediate coordinated RhoA-ROCK1 expression and signaling in breast cancer cells

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Breast cancers often contain regions of reduced O_2 availability, leading to increased activity of hypoxia-inducible factors (HIFs). Here, we demonstrate that HIFs activate transcription of the Rho family member *RHOA* and Rho kinase 1 (*ROCK1*) genes, leading to cytoskeletal changes that underlie the invasive cancer cell phenotype. ROCK1 is a kinase that regulates myosin light-chain activity, leading to actin-myosin contraction, which is the basis for cell movement. Coordinately increased levels of RhoA and ROCK1 mRNA in human breast cancers predicted patient mortality. These results (pp. E384–E393) demonstrate that a microenvironmental stimulus, hypoxia, can activate a critical signal transduction pathway, independent of genomic alterations, to drive cancer progression.

Contactin-1 regulates myelination and nodal/paranodal domain organization in the central nervous system

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Myelin is a multilayered membrane sheath that encircles axons to enable rapid information processing and protect neurons. Formation of myelin requires communication between axons and oligodendrocytes, the myelin-forming cells in the CNS. Here (pp. E394–E403) we identify Contactin-1 as a critical signal for axon–glia communication in CNS myelin. Gene ablation in mice shows that Contactin-1 is necessary for myelin sheath formation by oligodendrocytes and establishment of paranodal axoglial junctions that regulate the domain organization and enable rapid nerve impulse conduction of myelinated nerves. The multiple and critical aspects of Contactin-1 in central myelin formation identified in the current study will guide novel approaches aimed at enhancing regeneration in demyelinating diseases that specifically affect the CNS.

Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization

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Chitin perception by plant receptors triggers various defense responses important for plant immunity. We show (pp. E404–E413) the molecular basis of chitin recognition by the rice receptor, CEBiP (chitin-elicitor binding protein), and following receptor dimerization based on the results of biochemical studies, epitope mapping by saturation transfer difference NMR spectroscopy and molecular modeling/docking studies. These results clearly indicated that two CEBiP molecules simultaneously bind to one *N*-acetylchitoheptaose/octaose from the opposite side, through a binding site in the central lysin motif region, resulting in the dimerization of CEBiP. Based on these observations, we proposed a hypothetical model for the ligand-induced activation of a receptor complex, involving CEBiP and *Oryza sativa* chitin-elicitor receptor kinase 1 (OsCERK1).