PNAS Plus Significance Statements

Structural basis for specificity and promiscuity in a carrier protein/enzyme system from the sulfur cycle

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Certain metabolic pathways use a carrier protein to shuttle covalently attached intermediates between the active sites of enzymes. However, the details of the carrier protein–partner interactions have only been elucidated in a few cases. We have used biophysical methods and crystallography to obtain a molecular-level description of the interactions between a carrier protein and an enzyme involved in bacterial sulfur oxidation. Characterization of the contact sites between the two proteins suggests a basis for the promiscuous, but specific, binding interactions of the carrier protein. We also infer that the enzyme discriminates between the substrate- and product-bound forms of the carrier protein based on different interaction kinetics and link this behavior to a structural change at the enzyme active site. (See pp. E7166–E7175.)

cAMP-induced phosphorylation of 26S proteasomes on Rpn6/PSMD11 enhances their activity and the degradation of misfolded proteins

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This report describes an important new mechanism regulating protein degradation in mammalian cells through phosphorylation of the 26S proteasome. Treatments that raise cAMP and activate PKA cause phosphorylation of Rpn6/PSMD11, a subunit of the 19S regulatory complex. This modification enhances the proteasome's capacity to hydrolyze ubiquitinated proteins, ATP, and small peptides, and in cells stimulates the degradation of aggregation-prone proteins, including ones that cause neurodegenerative diseases (tau, SOD1, TDP43, and FUS). Our related collaborative study demonstrated that raising cAMP in brain promotes the clearance of aggregated tau in a mouse tauopathy model. This enhancement of proteasome activity and breakdown of misfolded proteins represents a new function of the cAMP/PKA pathway that offers a promising approach to treat proteotoxic diseases. (See pp. E7176–E7185.)

Kinetics of nucleotide-dependent structural transitions in the kinesin-1 hydrolysis cycle

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We use high spatiotemporal resolution single-molecule microscopy to directly visualize the structural transitions underlying each step of the molecular motor kinesin-1 at physiological stepping rates. Our results identify a one-head–bound intermediate in the stepping cycle that is initiated by ATP binding and is terminated by ATP hydrolysis. These results supersede previous functional studies because they identify the transitions that must occur to produce a step as opposed to transitions that may occur if the motor is studied under controlled conditions. We thus show that kinesin utilizes a two-step power-stroke mechanism to walk at maximum velocity. The single-molecule methods developed here are broadly applicable for resolving protein conformational changes as small as 2 nm with millisecond temporal resolution. (See pp. E7186–E7193.)

Timing of androgen receptor disruption and estrogen exposure underlies a spectrum of congenital penile anomalies

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Birth defects of external genitalia occur at a striking frequency, affecting ~1:250 live births. Congenital penile anomalies (CPAs) encompass a range of malformations, including failure of urethral tube closure (hypospadias), penile curvature (chordee), micropenis, and feminization of male genitalia. Both genetic anomalies and exposures to endocrine disrupting chemicals (EDCs) are suspected to be involved; however, little is known about the underlying causes or the developmental window(s) of sensitivity to anti-androgenic or estrogenic signals. This study shows that disruption of androgen signaling at different stages of genital development can induce different types of CPA. We identify a cell type in which the androgen receptor (AR) is essential for genital masculinization and uncover previously unknown mechanisms through which anti-androgenic and estrogenic signals induce penile malformations. (See pp. E7194–E7203.)

Evolution of stickleback in 50 years on earthquake-uplifted islands

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On several Alaskan islands, phenotypically variable threespine stickleback fish now live in ponds that were formed during uplift caused by the 1964 Great Alaska Earthquake. We analyzed phenotypic and genome-wide genetic divergence of resident freshwater and oceanic threespine stickleback populations from three islands. These data support the hypothesis that the freshwater populations evolved repeatedly from their oceanic ancestors in the past half-century, and have differentiated to nearly the same extent as populations that were founded thousands of years ago. This work raises the possibility that much of the evolution that occurs when oceanic stickleback invade fresh water takes place in fewer than 50 generations after colonization, rather than gradually over thousands of years. (See pp. E7204–E7212.)

Casein kinase II promotes target silencing by miRISC through direct phosphorylation of the DEAD-box RNA helicase CGH-1

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MicroRNAs (miRNAs) are critical regulators of diverse biological processes. Despite rapid advances in understanding miRNA biogenesis and function, a gap remains in our knowledge of how miRNA effector complex activity [miRNA-induced silencing complex (miRISC)] is modulated. Specifically, the importance of posttranslational protein modifications in controlling miRISC activity remains largely unexplored. Here, we characterize a previously unidentified role for the conserved serine/threonine kinase, casein kinase II (CK2), in promoting the miRNA pathway in *Caenorhabditis elegans*. Notably, we establish the requirement of CK2 for miRNA function and provide mechanistic evidence that loss of CK2 compromises miRISC binding to mRNA targets. Furthermore, we identify that the miRISC cofactor and DEAD-box RNA helicase, CGH-1/DDX6, is phosphorylated by CK2 at a conserved residue, which is required for CGH-1–mediated miRNA function. (See pp. E7213–E7222.)

Bovine *NK-lysin*: Copy number variation and functional diversification

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The cattle genome contains expanded families of several genes involved in innate immunity. A single copy of the *NK-lysin* gene is annotated in the genomes of most mammals, including humans, but this study identified a family of *NK-lysin* genes in cattle consisting of four functional members. Although this family mirrors the numerical expansion of other immune-related genes, including interferons, defensins, and cathelicidins, in the cattle genome, we also see a diversification of function exhibited by differential tissue expression in the gene family. The current state of this site in the bovine genome appears to capture the evolutionary transition from copy number variation to the fixation of novel gene function within a segmentally duplicated region. (See pp. E7223–E7229.)

Lymphomagenic CARD11/BCL10/MALT1 signaling drives malignant B-cell proliferation via cooperative NF-κB and JNK activation

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The activated B cell-like (ABC) subtype of diffuse large B-cell lymphoma (DLBCL) has a poor clinical outcome and is characterized by constitutive caspase recruitment domain-containing protein 11 (CARD11)/B-cell CLL/lymphoma 10 (BCL10)/mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1) signaling and nuclear factor kappa-B (NF-κB) activation, which is essential for tumor cell survival. However, NF-κB inhibitors have not reached the clinic due to high toxicity, and alternative strategies for targeted therapies are needed. Using mouse genetics, we demonstrate that forced CARD11/BCL10/MALT1 signaling drives lethal lymphoproliferation through simultaneous NF-κB and c-Jun N-terminal kinase (JNK) activation. Pharmacological inhibition of JNK was similar to NF-κB blockage and toxic to autonomously proliferating murine B cells, and constitutive JNK activity was observed in human ABC-DLBCL cells, which are also sensitive to JNK inhibitors. Our results identify the JNK pathway as a therapeutic target for DLBCL. (See pp. E7230–E7238.)

CD11b regulates obesity-induced insulin resistance via limiting alternative activation and proliferation of adipose tissue macrophages

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Obesity is associated with long-term low-grade inflammation characterized by the accumulation of adipose tissue macrophages (ATMs). One important molecule that regulates the migration of monocytes/macrophages is CD11b (integrin α_M). Here we show an unexpected role of CD11b in modulating the IL-4/STAT6 signaling in macrophages, thereby limiting IL-4/STAT6–mediated proliferation and alternative activation of ATMs. In the absence of CD11b, there is an increase in ATM in situ proliferation and an enhancement of alternatively polarized phenotypes. Importantly, the alternatively activated ATMs attenuate obesity-related insulin resistance in CD11b-deficient mice. These results reveal a previously unidentified physiological function of CD11b, which could be a therapeutic target for insulin resistance. (See pp. E7239–E7248.)

Specific induction of endogenous viral restriction factors using CRISPR/Cas-derived transcriptional activators

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Cells encode several effective antiviral proteins, yet these are sometimes not expressed in infected cells. Using a synthetic transcriptional activator based on the bacterial RNA-guided DNA binding protein Cas9, we demonstrate the efficient induction of two antiviral effectors, APOBEC3G (A3G) and APOBEC3B (A3B), in human cells that normally express neither protein. A3G is susceptible to degradation by the HIV-1 Vif protein, whereas A3B is resistant to Vif. As a result, only the induced A3B inhibited wild-type HIV-1 infectivity. However, both induced factors blocked the replication of a Vif-deficient HIV-1 mutant. These data demonstrate that Cas9-derived transcription factors can effectively induce human genes that regulate virus replication, thus setting the scene for their use in genomic screens to identify such factors. (See pp. E7249–E7256.)

5-hydroxymethylation of the EBV genome regulates the latent to lytic switch

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Epstein–Barr virus (EBV) normally establishes a lytic infection in differentiated epithelial cells. However, in the abnormal context of

nasopharyngeal carcinoma (NPC), EBV latently infects undifferentiated epithelial cells. Here we demonstrate that the EBV genome can become 5-hydroxymethylated and that this DNA modification affects EBV lytic reactivation. We find that 5-hydroxymethylcytosine accumulates during differentiation of normal epithelial cells but not in EBV+ NPCs. Furthermore, we show that ten–eleven translocation (TET) enzymes dysregulate lytic viral reactivation by altering the 5-methylcytosine and 5-hydroxymethylcytosine state of lytic promoters. These data suggest that loss of TET activity may promote cellular hypermethylation and alter EBV gene regulation in NPC tumors. (See pp. E7257–E7265.)

Glycan:glycan interactions: High affinity biomolecular interactions that can mediate binding of pathogenic bacteria to host cells

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Pathogens use cell surface carbohydrates as a means of attachment to host tissues. In several pathogenic bacteria, truncation of surface carbohydrates, lipooligosaccharide, or lipopolysaccharide have been reported to significantly reduce bacterial adherence to host cells. Here, we show that the lipooligosaccharide/lipopolysaccharide of four distinct bacterial pathogens bind directly to a range of host glycans. Surface plasmon resonance data confirmed binding among 66 different host–glycan:bacterial–glycan pairs. We also demonstrated that bacterial adherence can be competitively inhibited by either host cell or bacterial glycans. Our discovery of high-affinity glycan:glycan interactions in infectious disease may provide new approaches for therapy and prevention. The discovery of the existence of extensive, high-affinity interactions between glycans will alter the perception of the importance of these macromolecular interactions in all biological systems. (See pp. E7266–E7275.)

Antifungal drug itraconazole targets VDAC1 to modulate the AMPK/mTOR signaling axis in endothelial cells

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Tumors promote angiogenesis to facilitate their growth and metastasis; thus, inhibition of angiogenesis is a promising strategy for treating cancer. During angiogenesis, endothelial cells (EC) are stimulated by proangiogenic factors to proliferate and migrate, leading to the formation of new blood vessels. Understanding the mechanisms regulating EC function therefore is essential for the development of new antiangiogenic interventions. Here, we identify a novel mechanism of EC regulation by the recently discovered angiogenesis inhibitor itraconazole, mediated by direct binding to the mitochondrial protein voltage-dependent anion channel 1 (VDAC1). VDAC1 inhibition perturbs mitochondrial ATP production, leading to activation of the AMP-activated protein kinase pathway and subsequent inhibition of mechanistic target of rapamycin, a regulator of EC proliferation. This study suggests VDAC1 may serve as a new therapeutic target for angiogenesis inhibition. (See pp. E7276–E7285.)

KCNE3 acts by promoting voltage sensor activation in KCNQ1

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The association of KCNE3 beta subunits to KCNQ1 channels turns voltage-dependent KCNQ1 channels into apparent voltage-independent KCNQ1/KCNE3 channels that are important for the transport of water and salts across epithelial cell layers. Because KCNQ1/KCNE3 channels are necessary for water and salt secretion in the colon, KCNQ1/KCNE3 channels are a potential drug target in the treatment of secretory diarrhea. Mutations in KCNE3 have also been associated with diseases such as cardiac arrhythmia and tinnitus. We here propose a model for how KCNE3 turns KCNQ1 into a voltage-independent channel. Our model will allow for a better understanding of how mutations in KCNQ1 and KCNE3 cause diseases and how to design drugs to treat these diseases. (See pp. E7286–E7292.)

Methylome analysis reveals an important role for epigenetic changes in the regulation of the *Arabidopsis* response to phosphate starvation

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Significant progress has been achieved in our understanding of plant adaptive responses to ensure growth and reproduction in soils with low phosphate (Pi) availability. However, the potential role of epigenetic mechanisms in the modulation of these responses remains largely unknown. In this article, we describe dynamic changes in global DNA methylation patterns that occur in *Arabidopsis* plants exposed to low Pi availability; these changes are associated with the onset of Pi starvation responses. We show that the expression of a subset of low Pi-responsive genes is modulated by methylation changes and that DNA methylation is required for the proper establishment of developmental and molecular responses to Pi starvation. (See pp. E7293–E7302.)