PNAS Plus Significance Statements

Vascular disease-causing mutation R258C in *ACTA2* disrupts actin dynamics and interaction with myosin

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Point mutations in vascular smooth muscle α-actin are the most prevalent cause of familial thoracic aortic aneurysms leading to acute dissections, yet the molecular mechanism by which these mutations affect actin function is unknown. An underlying cause of the disease is thought to be contractile dysfunction, which initiates adaptive pathways to repair the defects in the smooth muscle cells. Here, we investigate the effects of the R258C mutation, a prevalent mutation in humans with a poor prognosis. The mutant actin shows multiple defects, including impaired interaction with myosin, formation of less stable filaments, and enhanced levels of monomer. These defects are likely to decrease cellular force production and initiate aberrant mechanosensing pathways that culminate in the disease. (See pp. E4168–E4177.)

CBR antimicrobials inhibit RNA polymerase via at least two bridge-helix cap-mediated effects on nucleotide addition

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The multisubunit RNA polymerases (RNAPs) are complex molecular machines that control catalysis through concerted conformational changes of conserved structural modules surrounding the active site. Central to these modules is the bridge helix (BH). The nature of these conformational changes and their detailed roles in the different steps of the RNAP nucleotide addition cycle are central issues in understanding the structural basis of RNAP catalytic activity. We report crystal structures of *Escherichia coli* RNAP complexes with a class of small molecule inhibitor (CBR inhibitors) and biochemical tests that establish two distinct effects of the inhibitors on the RNAP catalytic site. These results provide insights into the enzyme's catalytic mechanism. (See pp. E4178–E4187.)

Plasmid replication initiator interactions with origin 13-mers and polymerase subunits contribute to strand-specific replisome assembly

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Research on DNA replication initiation has not revealed the exact mechanism for replication complex de novo assembly at the origin or how the directionality of replication is determined. To date, no evidence for direct involvement of a replication initiation protein (Rep) in the process of polymerase recruitment has been reported. This work demonstrates that a plasmid Rep, in addition to its already described functions in origin opening and helicase recruitment, can serve as a DNA polymerase anchoring factor. Through its interaction with 13-mer sequences on one strand of initially unwound DNA and interactions with the subunits of DNA polymerase, the initiation protein facilitates strand-specific replisome assembly at the replication origin. This step determines the direction of DNA replication. (See pp. E4188–E4196.)

Structural basis for methyl-donor–dependent and sequence-specific binding to tRNA substrates by knotted methyltransferase TrmD

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In bacterial tRNAs with the ^{36}GG^{37} sequence, where positions 36 and 37 are, respectively, the third letter of the anticodon and 3′ adjacent to the anticodon, the modification of N^1-methylguanosine (m^1G) at position 37 prevents +1 frameshifts on the ribosome. The m^1G37 modification is introduced by the enzyme TrmD, which harbors a deep trefoil knot within the *S*-adenosyl-L-methionine (AdoMet)-binding site. We determined the crystal structure of the TrmD homodimer in complex with a substrate tRNA and an AdoMet analog. The structure revealed how TrmD, upon AdoMet binding in the trefoil knot, obtains the ability to bind the substrate tRNA, and interacts with G37 and G36 sequentially to transfer the methyl moiety of AdoMet to the N^1 position of G37. (See pp. E4197–E4205.)

Heterogeneous binding of the SH3 client protein to the DnaK molecular chaperone

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Heat shock protein 70 (Hsp70) molecular chaperones play key roles in protein folding and other cellular processes. The effect of Hsp70 on the conformation of its substrate proteins is still largely unknown. This study unveils, for the first time to our knowledge, the effect of the bacterial Hsp70 chaperone DnaK on the structure of the full-length substrate protein SRC homology 3 domain (SH3). We show that multiple largely unstructured conformations of SH3, distinct from the protein's unfolded state, interact with DnaK. The bound client protein shares a flexible N terminus and multiple slowly interconverting conformations in different parts of the sequence. In all, there is significant structural and dynamical heterogeneity. This result is important because it reveals that proteins may undergo conformational sampling while chaperone-bound. (See pp. E4206–E4215.)

The Xist RNA-PRC2 complex at 20-nm resolution reveals a low Xist stoichiometry and suggests a hit-and-run mechanism in mouse cells

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X-chromosome inactivation (XCI) is initiated by the long noncoding RNA Xist. Here we view Xist RNA and the Xi at 20-nm resolution using STochastic Optical Reconstruction Microscopy (STORM) and observe dynamics at the single-cell level not predicted by epigenomic analysis. Only 50–100 Xist molecules and ∼50 PRC2 foci are observed per Xi, contrasting with the chromosome-wide "coat" observed by deep sequencing and conventional microscopy. Xist knock-off experiments enable visualization of

dissociation and relocalization dynamics, and support a functional tethering of Xist and PRC2. Thus, Xist-PRC2 complexes are less numerous than expected, implying that the Xist-PRC2 complexes methylate nucleosomes in a hit-and-run model. (See pp. E4216–E4225.)

Knockout silkworms reveal a dispensable role for juvenile hormones in holometabolous life cycle

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The juvenile–adult transition is a key developmental process in organisms. A long-held paradigm in insect endocrinology is that juvenile hormones (JHs) prevent metamorphosis until larvae attain an appropriate size for the juvenile–adult transition. However, little is known about the roles for JHs during embryonic and very early larval stages. We established knockouts of the silkworm, a classic model insect, and show that embryogenesis and maintenance of juvenile status during the early larval stages are largely independent of JHs or the JH-signaling pathway. Our results also suggest that an unidentified factor or signal is required to acquire the competence for metamorphosis. The presence of this factor has long been overlooked because JHs may conceal its action. (See pp. E4226–E4235.)

Simultaneous deletion of the methylcytosine oxidases Tet1 and Tet3 increases transcriptome variability in early embryogenesis

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Development of preimplantation embryos entails global DNA demethylation on the zygotic genome. The original thought was that TET-deficient embryos would be unlikely to survive early embryogenesis because they would be unable to mediate genome-wide demethylation in the zygote and preimplantation embryo. However, mice lacking the individual TET proteins Tet1, Tet2, or Tet3 have survived until birth and beyond, suggesting redundancy among TET proteins in the early embryogenesis. Here we report that preimplantation embryos doubly disrupted for *Tet1* and *Tet3* show abnormal embryonic phenotypes, whose incomplete penetrance correlates with a high variability of transcriptional profiles and DNA methylation status. Our data suggest that in addition to facilitating DNA demethylation, TET proteins and oxidized methylcytosines may regulate the consistency of gene transcription during embryogenesis. (See pp. E4236–E4245.)

RNA helicase HEL-1 promotes longevity by specifically activating DAF-16/FOXO transcription factor signaling in *Caenorhabditis elegans*

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RNA helicases are a large family of enzymes that regulate the generation and maintenance of RNA. However, the physiologic roles of RNA helicases in animal aging remained unknown. Here we show that an RNA helicase, helicase 1 (HEL-1), extends the lifespan of the roundworm *Caenorhabditis elegans* by up-regulating the longevity transcription factor forkhead box O (FOXO). Our finding suggests that an RNA helicase can have rather specific roles in animal longevity. A number of studies show that variants of FOXO are linked to human aging and longevity. In addition, the mammalian HEL-1 homolog has been implicated in cellular aging. Thus, our work may have direct implications in mammalian aging, and the human HEL-1 homolog may work with FOXO to increase lifespan. (See pp. E4246–E4255.)

Dengue virus infection elicits highly polarized CX3CR1$^+$ cytotoxic CD4$^+$ T cells associated with protective immunity

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Infections with any of the four dengue virus serotypes (DENV 1–4) are the most prevalent and rapidly spreading mosquito-borne viral infections in humans. There is no treatment or vaccine currently available. We found that the virus-specific cells display a highly polarized cytotoxic phenotype that correlated with expression of a protective HLA DR allele. Although the occurrence of cytotoxic CD4$^+$ T cells in humans has been described in the context of some chronic viral infections, to our knowledge, this is the first report of ex vivo cytotoxic CD4$^+$ activity after exposure with an acute virus. These results will help shed light on the specific role of CD4$^+$ T cells in DENV infection and may help in finding a correlate of protection. (See pp. E4256–E4263.)

Protein tyrosine phosphatase SAP-1 protects against colitis through regulation of CEACAM20 in the intestinal epithelium

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Much attention has been recently paid to the role of intestinal epithelial cells in the homeostatic regulation of intestinal immunity. Here we show that ablation of stomach-cancer–associated protein tyrosine phosphatase 1 (SAP-1) markedly increased the severity of colitis in interleukin (IL)-10–deficient mice, suggesting that SAP-1 protects against colitis in a cooperative manner with IL-10. We also identify carcinoembryonic antigen-related cell adhesion molecule (CEACAM) 20, an intestinal microvilli-specific membrane protein, as a dephosphorylation target for SAP-1. Indeed, tyrosine phosphorylation of CEACAM20 promotes the binding of spleen tyrosine kinase (Syk) and activation of nuclear factor-κB (NF-κB), thereby inducing production of chemokines such as IL-8. Thus, we propose a mechanism by SAP-1 and CEACAM20 in the intestinal epithelium for regulation of the intestinal immunity. (See pp. E4264–E4271.)

Keap1 regulates inflammatory signaling in *Mycobacterium avium*-infected human macrophages

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Inflammatory signaling is a central mechanism controlling host defenses to pathogens. Members of *Mycobacterium avium* complex cause disease in immunocompromised patients and in individuals with predisposing lung abnormalities. We provide evidence of a mechanism in human primary macrophages whereby the oxidative stress sensor Kelch-like

ECH-associated protein 1 (Keap1) negatively regulates inflammatory responses and thus facilitates intracellular growth of *M. avium*. Our findings are of high biological and clinical significance, as opportunistic infections with nontuberculous mycobacteria are receiving renewed attention because of increased incidence and difficulties in treatment. Altered Keap1 gene expression may also have vital clinical implications for other inflammation-associated conditions, opening novel research venues for translational research, for instance in the expanding field of host-targeted therapy for infectious diseases. (See pp. E4272–E4280.)

Small-molecule enhancers of autophagy modulate cellular disease phenotypes suggested by human genetics

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Given the importance of autophagy in a number of human diseases, we have identified small-molecule modulators of autophagy that affect disease-associated phenotypes in relevant cell types. BRD5631 and related compounds can serve as tools for studying how autophagy regulates immune pathways, and for evaluating the therapeutic potential of modulating autophagy in a variety of disease contexts. Deeper investigation into their mechanisms of action may reveal proteins and pathways that could serve as relevant targets for future therapeutic discovery. (See pp. E4281–E4287.)

MicroRNA-224 promotes tumor progression in nonsmall cell lung cancer

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Aberrant microRNA (miRNA) expression is involved in tumorigenesis, and *miR-224* was observed to be up-regulated in certain tumor types. However, the role of *miR-224* in the pathogenesis of lung cancer remains poorly understood. Here, we comprehensively analyzed and revealed mechanisms of *miR-224* up-regulation and its oncogenic role in nonsmall cell lung cancer (NSCLC). We showed that *miR-224* promotes cellular migratory, invasive, and proliferative capacity and tumor growth both in vitro and in vivo. Furthermore, we identified TNFα-induced protein 1 and SMAD4 as targets of *miR-224*. In addition, up-regulated *miR-224* expression in NSCLC is partially controlled by its promoter region's hypomethylation and activated ERK signaling. Our finding suggests that targeting *miR-224* might be a promising therapeutic strategy in the treatment of NSCLC. (See pp. E4288–E4297.)

Entner–Doudoroff pathway for sulfoquinovose degradation in *Pseudomonas putida* SQ1

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Phototrophic organisms worldwide produce estimated 10 gigatons of sulfoquinovose (SQ) per year; hence, complete degradation of SQ by bacteria is an important part of the biogeochemical sulfur cycle. Here, we show that *Pseudomonas putida* SQ1 catabolizes SQ to 3-sulfolactate (SL) in analogy to the Entner–Doudoroff pathway for glucose-6-phosphate, involving five newly discovered reactions, enzymes, and genes, and three newly discovered organosulfur intermediates. The SL can be mineralized by other bacteria, thus closing the sulfur cycle within a bacterial community. The genes for the SQ Entner–Doudoroff pathway can be found in genomes of a wide range of Proteobacteria, which shows that SQ utilization is a widespread and important, but still underrecognized, trait of bacteria in all environments where SQ is produced and degraded. (See pp. E4298–E4305.)

Modulation of the cGAS-STING DNA sensing pathway by gammaherpesviruses

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Kaposi's sarcoma-associated herpesvirus (KSHV) is a DNA virus that is linked to several human malignancies. The cGMP-AMP synthase (cGAS) and stimulator of interferon genes (STING) pathway is able to detect KSHV during primary infection and regulates the reactivation of KSHV from latency. We screened KSHV proteins for their ability to inhibit this pathway and block IFN-β activation. One KSHV protein, vIRF1, inhibited this pathway by preventing STING from interacting with TBK1 and inhibiting STING's phosphorylation and concomitant activation. Moreover, depletion of vIRF1 in the context of KSHV infection prevented efficient viral reactivation and replication, and increased the host IFN response to KSHV. Collectively, our results demonstrate that the modulation of this pathway is important for viral transmission and the lifelong persistence of gammaherpesviruses in the human population. (See pp. E4306–E4315.)

Structural elements that underlie Doc2β function during asynchronous synaptic transmission

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Evoked synaptic transmission is mediated by synchronous and asynchronous phases of neurotransmitter release. Synaptotagmin 1 (syt1) serves as the Ca^{2+} sensor for synchronous release. Recently, we proposed that double C2-like domain-containing protein alpha and beta (Doc2α and Doc2β), cytosolic proteins with tandem C2 domains homologous to syt1, function as Ca^{2+} sensors for asynchronous release, but this idea remains controversial. Here, we systematically analyzed the functional significance of each Ca^{2+} ligand in Doc2β and found a correlation between the Ca^{2+}-dependent translocation activity of these mutants (to the plasma membrane) and changes in asynchronous release. Moreover, we show that syt1–Doc2β chimeras exhibit altered kinetics in vitro and change the rates of synaptic transmission in cultured neurons. These results establish Doc2β as a Ca^{2+} sensor for the slow phase of neurotransmission. (See pp. E4316–E4325.)

Replacing SNAP-25b with SNAP-25a expression results in metabolic disease

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Our changed lifestyle, including decreased physical activity and increased food consumption, is leading to a pandemic of obesity and type 2 diabetes, the metabolic syndrome. Impaired release of hormones, such as insulin, from excitable cells usually is considered a symptom, not a cause, of this syndrome. Here, however, we show that a small genetic modification, replacing synaptosomal-associated protein of 25 kDa (SNAP-25), SNAP-25b with SNAP-25a in the machinery mediating stimulus-dependentrelease of hormones and neurotransmitters is sufficient to provoke hypothalamic dysfunction, obesity, and type 2 diabetes. When combined with a Western diet, this genetic condition triggers severe diabesity. Our work expands previous knowledge supporting the notion that many individuals have an increased susceptibility to developing metabolic disease because of a genetic predisposition. (See pp. E4326–E4335.)