PNAS Plus Significance Statements

Lipid topology and electrostatic interactions underpin lytic activity of linear cationic antimicrobial peptides in membranes

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We propose a mechanistic framework that explains the activity and selectivity of an important class of compounds known as linear cationic antimicrobial peptides. These molecules have the potential to be developed into highly potent and selective pharmaceuticals, as they are able to discriminate between mammalian and bacterial membranes, and so destroy pathogens. By comparing both selective and nonselective peptides, we show that their activity is governed by topological and electrostatic interactions between the membrane-bound peptide and the surrounding lipids. This framework could underpin strategies for the rational design of therapeutic agents that are potentially able to bypass the mechanisms of acquired bacterial drug resistance. (See pp. E8324–E8332.)

Evolutionary diversification of protein–protein interactions by interface add-ons

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Proteins adopt no more than a thousand folds, and the number of different protein–protein interface geometries is restricted to around 1,000, too. Given this limited structural repertoire, it has remained elusive how hundreds of thousands of specific protein–protein interactions evolved and how unspecific interactions are avoided. We report on a strategy to solve this dilemma, which is the integration of additional structural elements at the interface periphery that guarantee specificity. We named these elements "interface add-ons" to reflect the benefit they provide to protein interfaces, as software add-ons do to web browsers or as additional bits turn a master key into a special key. (See pp. E8333–E8342.)

Asymmetric mechanosensitivity in a eukaryotic ion channel

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One important way in which living organisms are able to detect and respond to their environment is via the conversion of mechanical forces into electrical signals. However, the molecular mechanisms that enable mammalian "mechanosensitive" ion channels to detect a wide profile of forces within the membrane remain unclear. By studying the functional activity of individual TREK-2 K2P channels inserted in different directions into a lipid bilayer, we are now able to describe how the asymmetric structure of this channel enables it to sense such a broad profile of forces. These results help us understand how eukaryotic ion channels respond to a rich variety of sensory stimuli. (See pp. E8343–E8351.)

Sirt7 promotes adipogenesis in the mouse by inhibiting autocatalytic activation of Sirt1

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This paper describes a mechanism of regulation of Sirt1 activity by Sirt7. Deacetylases Sirt1 and Sirt7 belong to the mammalian family of seven sirtuins, which play important regulatory roles in several biological processes such as metabolism and aging. We discovered that Sirt1 is able to augment its own catalytical activity by autodeacetylation. Sirt7 binds to Sirt1 and inhibits its activity. The biological importance of this regulation was revealed in the differentiation and maintenance of white adipose tissue (WAT). Sirt7 knockout mice contain a significantly diminished amount of WAT due to the increased Sirt1 activity and Sirt1 inhibition restores WAT in Sirt7 knockout mice. (See pp. E8352–E8361.)

Comparative analysis reveals genomic features of stress-induced transcriptional readthrough

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Cells and organisms live in constantly changing environments. Therefore, cells have evolved complex mechanisms to cope with physiological and environmental stresses. Many of these mechanisms involve transcriptional responses facilitating survival and adaptation. Recent evidence documents extensive transcriptional readthrough beyond annotated gene ends in response to stress, but the role and regulation of these downstream of gene-containing transcripts (DoGs) remain elusive. Here we report that induction of transcriptional readthrough is a hallmark of the mammalian stress response. We explore its causes and consequences in a genome-wide fashion, identifying thousands of readthrough transcripts that are induced in three different stress conditions. Our results suggest potential roles for this class of transcripts in the maintenance of open chromatin under stress. (See pp. E8362–E8371.)

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Functional screening in human cardiac organoids reveals a metabolic mechanism for cardiomyocyte cell cycle arrest

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Engineered cardiac muscle can be used to promote the structural and functional maturation of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs). However, previous studies have not yet produced cardiac tissues with metabolic and proliferative maturation. Here, we develop a 96-well screening platform and screen for cardiac maturation conditions in engineered cardiac muscle. We found that simulating the postnatal switch in metabolic substrates from carbohydrates to fatty acids promoted a switch in metabolism, DNA damage response, and cell cycle arrest in hPSC-CM. Our study shows that this mechanism can be harnessed to enhance the maturation of human hPSC-CM and cardiac tissues, which has major implications for stem cell sciences, drug discovery, and regenerative medicine. (See pp. E8372–E8381.)

Small genome symbiont underlies cuticle hardness in beetles

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Beetles are successful in the terrestrial ecosystem, which is attributable to, at least partly, their highly sclerotized exoskeleton. Here, we report a bacterial symbiont extremely specialized for underpinning the beetle's hardness. The ancient endosymbiont *Nardonella* associated with weevils has an extremely small genome devoted to a single biological function, tyrosine provisioning, which is needed for insect's cuticle formation and hardening. Notably, only the final step reaction of the tyrosine synthesis pathway is complemented by host-encoded aminotransferases up-regulated in the bacteriome, highlighting a highly focused aspect of the host–symbiont metabolic integrity. Both symbiont suppression by an antibiotic and RNA interference of the host aminotransferases induce reddish and soft weevils, verifying the pivotal role of the symbiosis for the beetle's hardness. (See pp. E8382–E8391.)

Cytosine deamination and base excision repair cause R-loop–induced CAG repeat fragility and instability in *Saccharomyces cerevisiae*

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R-loops form when transcribed RNA remains bound to its DNA template to form a stable RNA:DNA hybrid. Stable R-loops at a CAG/CTG repeat tract, a sequence that can expand to cause human disease, cause DNA breaks as well as repeat instability. We found that R-loop–induced deamination of cytosines followed by base excision repair is responsible for causing CAG repeat breaks and contractions. Intriguingly, R-loop–dependent double-strand breaks were caused by the MutL gamma endonuclease, which is known to recognize structured DNA and cause nicks, defining a new mechanism for how R-loops can generate DNA breaks. Our results have implications for human repeat expansion diseases and provide a paradigm for how RNA:DNA hybrids can cause genome instability at structure-forming DNA sequences. (See pp. E8392–E8401.)

[PSI+] prion propagation is controlled by inositol polyphosphates

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The [PSI+] prion of baker's yeast is a filamentous polymer (amyloid) of the Sup35 protein, producing readthrough of translation termination and different degrees of growth slowing, depending on the prion variant. We show that certain inositol polyphosphates and pyrophosphates promote the propagation of the [PSI+] prion and that an inositol pyrophosphate pyrophosphatase has an antiprion effect. Inositol poly-/pyrophosphates are intracellular signaling molecules not previously connected with any amyloidosis. (See pp. E8402–E8410.)

IgH isotype-specific B cell receptor expression influences B cell fate

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B cells produce antibodies in the context of immunoglobulin heavy chain (IgH) isotypes (e.g., IgM, IgG, and IgE). Each of these is generated either as secreted proteins or as membrane-bound B cell antigen receptors (BCRs). While much is known about how IgH isotype dictates effector function of soluble antibodies, the role of antibody isotype in the context of BCRs is not well defined. Here we demonstrate that the membrane-bound versions (mIg) of IgM, IgG1, and IgE are produced from their natural genomic loci in a hierarchal fashion, where mRNA transcripts for mIgM are always more dominant than mIgG1, which are always more dominant than mIgE, regardless of cell stage. These isotype-specific expression differences contribute to B cell regulation. (See pp. E8411–E8420.)

MIF and D-DT are potential disease severity modifiers in male MS subjects

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The biological processes that are involved in the progression of multiple sclerosis (MS) are far from complete. Macrophage migration inhibitory factor (MIF) and its homolog, D-dopachrome tautomerase (D-DT), are immunoregulatory cytokines known to be involved in the worsening of various autoimmune disorders. We demonstrate that genetically controlled high MIF expression (and D-DT) promotes MS progression in males, suggesting that these two factors are sex-specific disease modifiers. In addition, we show that MIF or D-DT deficiency ameliorates the disease severity of the murine model of MS. Our data suggest that targeting CD74, the common receptor for MIF and D-DT, with therapies such as partial MHC class II constructs could be therapeutically beneficial for inhibiting MS clinical progression in selected patients. (See pp. E8421–E8429.)

IL-4–producing B cells regulate T helper cell dichotomy in type 1- and type 2-controlled diseases

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Cutaneous leishmaniasis and schistosomiasis are neglected tropical diseases for which there are no effective vaccines and

limited treatment strategies. To develop vaccine and therapeutic alternatives, a detailed understanding of host immunity is essential. We show a role for IL-4Rα–responsive B cells in host susceptibility to *Leishmania major* and protection against *Schistosoma mansoni* infection through the production of early IL-4, which in turn regulates Th2 cell polarization and disease outcome in mice. These important findings highlight the significant impacts that B cell-specific IL-4Rα and IL-4 responsiveness have in the context of type 1 (*L. major*) and type 2 (*S. mansoni*) pathogens. Thus, vaccine and therapeutic development should aim to target both B and T cell immunity for optimal efficacy. (See pp. E8430–E8439.)

Immunoreceptor tyrosine-based inhibitory motif–dependent functions of an MHC class I-specific NK cell receptor

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Natural killer (NK) cells are cytotoxic immune cells that are regulated by inhibitory receptors, such as murine Ly49s, that bind to MHC class I (MHC-I). Cancer immunotherapies are currently in clinical trial that target inhibitory NK-cell receptors analogous to checkpoint inhibitors that block inhibitory receptors on T cells. To improve checkpoint blockade of NK cells, it is critical to better understand the in vivo functions of inhibitory NK-cell receptors. Here, we developed a knock-in mouse with a targeted mutation predicted to abolish the signaling motif of the inhibitory receptor Ly49A. This mutant mouse revealed multiple mechanisms by which inhibitory receptor signaling controls NK-cell self-tolerance that could impact the efficacy of checkpoint blockade of NK cells. (See pp. E8440–E8447.)

Direct engagement of the PI3K pathway by mutant KIT dominates oncogenic signaling in gastrointestinal stromal tumor

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Oncogenic receptor tyrosine kinases (RTKs) are important drug targets in the clinical setting. While RTK inhibitors have become important tools in the clinic, as has been demonstrated with chronic myelogenous leukemia and gastrointestinal stromal tumors (GIST), drug resistance invariably develops. The tools and rationale for the treatment of RTK drug resistance are limited, and success is of short duration. The identification of secondary intracellular drug targets is thus of critical importance. By using new Kit-GIST mouse models in which specific Kit signaling cascades are inhibited, we show the importance of PI3 kinase signaling in tumor development, as well as the utility of PI3 kinase inhibition in the treatment of primary and imatinib-resistant GIST. These studies provide a rationale for targeting dominant molecular pathways in tumors driven by oncogenic kinases. (See pp. E8448–E8457.)

NCoR1-independent mechanism plays a role in the action of the unliganded thyroid hormone receptor

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Thyroid hormone receptors (TRs) mediate the genomic actions of thyroid hormones. In the absence of T3 (3,3′,5-triiodothyronine), the TR recruits a multiprotein repressor complex that decreases histone acetylation in the vicinity of target genes. Nuclear receptor corepressor 1 (NCoR1) is hypothesized to be the main corepressor that interacts with TR. Here we report that the deletion of NCoR1 does not prevent all gene repression and histone deacetylation across a variety of mouse models, whereas only the lack of TR was able to overturn the effects of hypothyroidism. Thus, we conclude that NCoR1 is not sufficient to mediate the actions of the unliganded TR; furthermore, our data suggest that alternative mechanisms of repression may be involved in the action of TRs. (See pp. E8458–E8467.)

Defective decidualization during and after severe preeclampsia reveals a possible maternal contribution to the etiology

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We provide evidence of a decidualization defect in the endometrium of women with severe preeclampsia (PE) that was detected at the time of delivery and persisted years after the affected pregnancy. We went on to link this defect to impaired cytotrophoblast invasion. The transcriptional signature of the defect could enable its detection before (or after) conception, which would aid the development of therapies focused on improving decidualization and perhaps preventing severe PE. (See pp. E8468–E8477.)

Endothelial insulin receptors differentially control insulin signaling kinetics in peripheral tissues and brain of mice

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Circulating hormones must cross the vascular endothelium to elicit their actions in target tissues via either transcytosis or paracellular diffusion. Insulin receptors on endothelial cells are believed to mediate transcytosis of circulating insulin, but how this affects insulin action in vivo is unknown. Here, we demonstrate that knockout of insulin receptors on endothelial cells delays the kinetics of activation of insulin signaling in skeletal muscle, fat, and several regions of the brain but not in liver or olfactory bulb. This alters the kinetics of insulin action in vivo and induces tissue-specific insulin resistance leading to dysregulated glucose and body weight homeostasis. (See pp. E8478–E8487.)

Host modification of a bacterial quorum-sensing signal induces a phenotypic switch in bacterial symbionts

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Specific bacterial communities colonize epithelial surfaces of most animals. In the last years many host mechanisms that control bacterial community composition have been identified. In contrast, only a few mechanisms are known that allow the host to target the behavior of its bacterial colonizers. We identified a eukaryotic mechanism based on an oxidoreductase activity, which enables the cnidarian *Hydra* to modify bacterial quorum-sensing (QS) molecules. The modification of QS signals leads to a phenotypic switch in the bacterial symbionts and promotes colonization of host tissue. (See pp. E8488–E8497.)

Leaderless secreted peptide signaling molecule alters global gene expression and increases virulence of a human bacterial pathogen

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Regulation of virulence factor production is critical for bacterial pathogenesis. The human pathogen group A *Streptococcus* (GAS) produces a potent secreted protease, streptococcal pyrogenic exotoxin B (SpeB), that is crucial for pathogenesis. Although it is known that GAS produces SpeB at high population density, the molecular mechanism whereby GAS coordinates temporal SpeB production is unknown. Here, we identify a GAS-encoded short leaderless intercellular peptide signal [SpeB-inducing peptide (SIP)], and define the mechanism by which SIP induces population-wide SpeB production and contributes to GAS virulence. Furthermore, discovery of SIP provides a framework for the identification of SIP-like leaderless peptide signals in other microorganisms. Thus, our data reveal a paradigm of bacterial signaling and identify previously unknown molecules that may serve as therapeutic targets. (See pp. E8498–E8507.)

Identification of sialic acid-binding function for the Middle East respiratory syndrome coronavirus spike glycoprotein

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Middle East respiratory syndrome coronavirus (MERS-CoV) recurrently infects humans from its dromedary camel reservoir, causing severe respiratory disease with an ∼35% fatality rate. The virus binds to the dipeptidyl peptidase 4 (DPP4) entry receptor on respiratory epithelial cells via its spike protein. We here report that the MERS-CoV spike protein selectively binds to sialic acid (Sia) and demonstrate that cell-surface sialoglycoconjugates can serve as an attachment factor. Our observations warrant further research into the role of Sia binding in the virus's host and tissue tropism and transmission, which may be influenced by the observed Sia-binding fine specificity and by differences in sialoglycomes among host species. (See pp. E8508–E8517.)

Exceptionally tight membrane-binding may explain the key role of the synaptotagmin-7 C_2A domain in asynchronous neurotransmitter release

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Synaptotagmins-1 and -7, act as Ca^{2+} sensors for the fast and slow components of neurotransmitter release, respectively, through the two C_2 domains that form their cytoplasmic region. Surprisingly, Ca^{2+}-binding to the synaptotagmin-7 C_2A domain is more critical for slow release than Ca^{2+}-binding to the C_2B domain, whereas the opposite was found for the synaptotagmin-1 C_2 domains and fast release. This paper suggests an explanation for this apparent contradiction, showing that the C_2A domain dominates binding of synaptotagmin-7 to membranes, whereas such binding is dominated by the C_2B domain in the case of synaptotagmin-1. Thus, membrane affinity may be a key determinant of the relative functional importance of synaptotagmin C_2 domains. (See pp. E8518–E8527.)

BPM-CUL3 E3 ligase modulates thermotolerance by facilitating negative regulatory domain-mediated degradation of DREB2A in *Arabidopsis*

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DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN 2A (DREB2A) is a key transcription factor for plant adaptation to drought and heat. DREB2A activity is strictly regulated via proteolysis mediated by the negative regulatory domain (NRD), although the molecular basis for this regulation has remained unclear for a decade. We reveal that BTB/POZ AND MATH DOMAIN proteins (BPMs), substrate adaptors for Cullin3-based E3 ubiquitin ligase, are the long-sought factors responsible for NRD-dependent DREB2A degradation. Through DREB2A degradation, BPMs negatively regulate the heat stress response and prevent the adverse effects of excess DREB2A on plant growth. Furthermore, we found the BPM recognition motif in various transcription factors, implying a general contribution of BPM-mediated proteolysis to divergent cellular responses via an accelerated turnover of transcription factors. (See pp. E8528–E8536.)

Exposure to seismic air gun signals causes physiological harm and alters behavior in the scallop *Pecten fumatus*

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Seismic surveys are used around the world as the primary means to explore for oil and gas deposits. Almost nothing is known regarding the impact of these sound signals on marine invertebrates. In this study, the physiological and behavioral effects of exposure on a commercially important bivalve, the scallop, were quantified. Following a field-based air gun exposure regime, exposed scallops were found to have significantly increased mortality rates; disrupted behavioral patterns and reflex responses, both during and following exposure; and altered hemolymph biochemistry, physiology, and osmoregulation capacity. These results indicate that air gun exposure has a harmful impact on scallops and raises concern over the impact on bivalves, due to their global ecological and economic importance. (See pp. E8537–E8546.)