PNAS Plus Significance Statements

IFI16 senses DNA forms of the lentiviral replication cycle and controls HIV-1 replication

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HIV-1 is a lentivirus and replicates through a replication cycle involving several DNA forms including ssDNA. Here we report that synthetic DNA oligos corresponding to DNA forms of the lentivirus replication cycle as well as viral DNA are detected by the immunological DNA sensor IFN-inducible protein 16 (IFI16) and stimulate innate immune responses through a pathway dependent on stimulator of IFN genes (STING). Moreover, we show (pp. E4571–E4580) that replication of HIV-1 is elevated in cells with decreased expression of IFI16 or STING. We suggest IFI16 is a sensor for lentivirus DNA in macrophages stimulating innate immune responses, which contribute to early control of the virus.

Microphysical effects determine macrophysical response for aerosol impacts on deep convective clouds

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Deep convective clouds (DCCs) play a key role in atmospheric circulation and the hydrological and energy cycle. How aerosol particles affect DCCs is poorly understood, making it difficult to understand current and future weather and climate. Our work (pp. E4581–E4590) showed that in addition to the invigoration of convection, which has been unanimously cited for explaining the observed results, the microphysical effects induced by aerosols are a fundamental reason for the observed increases in cloud fraction, cloud top height, and cloud thickness in the polluted environment, even when invigoration is absent. The finding calls for an augmented focus on understanding the changes in stratiform/anvils associated with convective life cycle.

Quantifying the dynamic interactions between a clathrin-coated pit and cargo molecules

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Clathrin-mediated endocytosis is the primary pathway of cargo internalization in mammalian cells. However, little is known about the time-dependent interactions between the endocytic machinery and cargo molecules. Nevertheless, these interactions are known to regulate the maturation of a clathrin-coated pit. In this study (pp. E4591–E4600), we attain a quantitative understanding of the interactions between clathrin-coated pits and cargo using a combination of imaging techniques, single-molecule tracking, and stochastic modeling. We observe that the binding times of cargo molecules are much shorter than the overall endocytic process, albeit they exhibit a very broad distribution. Our modeling explains the measured statistics of cargo captures and binding times. This work further identifies a mechanism for the large diversity in the dynamic behavior of clathrin structures.

Structural and genetic analyses reveal the protein SepF as a new membrane anchor for the Z ring

Ramona Duman, Shu Ishikawa, Ilkay Celik, Henrik Strahl, Naotake Ogasawara, Paulina Troc, Jan Löwe, and Leendert W. Hamoen

A key step in bacterial cell division is the polymerization of FtsZ at midcell into a ring-like structure. This so-called Z ring forms a scaffold for the other cell division proteins. FtsA anchors the Z ring to the cell membrane; however, many bacterial species do not have FtsA. Here (pp. E4601–E4610), we show that the conserved protein SepF, which forms large protein rings, also functions as a membrane anchor for the Z ring. We determined the molecular structure of the FtsZ-binding and ring-forming domain of SepF and show that the membrane-binding domain is located at the very beginning of the protein. These results explain why FtsA and SepF can fulfill similar functions in bacterial cell division.

Crystal structure of the human eIF4AIII–CWC22 complex shows how a DEAD-box protein is inhibited by a MIF4G domain

Gretel Buchwald, Steffen Schüssler, Claire Basquin, Hervé Le Hir, and Elena Conti

The fate of eukaryotic mRNAs is intimately linked to the complement of proteins that associate with them to form mRNA—protein complexes, the so-called messenger ribonucleoprotein particles (mRNPs). Transitions in the architecture of an mRNP lead to specific functional consequences. DEAD-box proteins are key players in orchestrating these structural rearrangements: They associate with RNA in response to ATP binding and dissociate from it upon ATP hydrolysis. In this paper, we have elucidated the molecular mechanisms by which a DEAD-box protein, which in human cells marks spliced mRNPs for a specialized surveillance pathway, is recognized by the MIF4G domain of a splicing factor. This structure (pp. E4611–E4618) shows how a MIF4G domain can act as a negative regulator of DEAD-box ATPase activity.

Growth hormone prevents the development of autoimmune diabetes

Ricardo Villares, Dimitri Kakabadse, Yasmina Juarranz, Rosa P. Gomariz, Carlos Martínez-A, and Mario Mellado

Although the relationship between endocrine and immune systems is well documented, few studies have been performed on autoimmune disorders other than those that are sex hormone-related. We studied a murine model of autoimmune diabetes, showing that growth hormone (GH) modifies the immune response to render diabetic mice resistant to disease development. The mechanism involves a GH-mediated effect on β-cell survival and/or proliferation and a direct effect on immune cells. GH triggers a cytokine environment that promotes anti-inflammatory macrophage polarization, maintains the activity of the suppressor T cells, and limits Th17 cell plasticity. This study (pp. E4619–E4627) provides evidence of the importance of endocrine control of immune functions and indicates that therapies based on GH analogs should be considered for treatment of autoimmune diabetes.

Peripheral subnuclear positioning suppresses *Tcrb* recombination and segregates *Tcrb* alleles from RAG2

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Eukaryotic genes are directed to distinct subnuclear compartments to regulate their activity. We show (pp. E4628–E4637) that different regions of the murine T-cell receptor β (*Tcrb*) locus interact independently with the nuclear lamina and that these interactions locally suppress the recombination of variable, diversity, and joining gene segments. This suppression is associated with the physical segregation of the locus from the recombinase protein, recombination-activating gene 2. Allelically excluded recombination of antigen receptor genes promotes the development of lymphocytes that each express a single antigen receptor. We propose that interaction with the nuclear lamina contributes to allelic exclusion by reducing the frequency of recombination of *Tcrb* alleles.

Structure and assembly of an inner membrane platform for initiation of type IV pilus biogenesis

Vijaykumar Karuppiah, Richard F. Collins, Angela Thistlethwaite, Ya Gao, and Jeremy P. Derrick

Type IV pili are long, thin fibers, formed mainly of polymers of a single pilin protein, which are displayed on the surfaces of many bacteria, including several human pathogens. Here, we report three-dimensional reconstructions of the PilMNO inner membrane complex, alone and in complex with pilin protein, through a combination of X-ray crystallography and electron microscopy. PilMNO forms a dimeric T-shaped structure, binding two copies of the pilin protein at its extremities. The results (pp. E4638–E4647) provide a structural model for the way in which pilin is harvested from the inner membrane and made available to other components of the type IV pilus biogenesis machinery.

Phosphorylation-dependent derepression by the response regulator HnoC in the *Shewanella oneidensis* nitric oxide signaling network

Lars Plate and Michael A. Marletta

The majority of response regulators in bacterial two-component signaling systems function as transcription factors to induce changes in gene expression in response to an external stimulus. Phosphorylation typically promotes subunit oligomerization, which enhances DNA binding. Here (pp. E4648–E4657) we describe a response regulator, HnoC, with an unprecedented regulation mechanism. Unphosphorylated HnoC exists as a tetramer and associates tightly to DNA, whereas phosphorylation causes subunit dissociation and transcriptional derepression. HnoC is part of a multicomponent signaling network, which controls biofilm formation in response to nitric oxide, possibly as a defense mechanism against NO cytotoxicity. HnoC represses transcription of all of the genes in the NO-signaling network, thus creating a transcriptional feedback loop, which could further tune the signaling dynamics.

Global methylation state at base-pair resolution of the *Caulobacter* genome throughout the cell cycle

Jennifer B. Kozdon, Michael D. Melfi, Khai Luong, Tyson A. Clark, Matthew Boitano, Susana Wang, Bo Zhou, Diego Gonzalez, Justine Collier, Stephen W. Turner, Jonas Korlach, Lucy Shapiro, and Harley H. McAdams

Caulobacter crescentus, a bacterium with an inherent asymmetric cell division, uses dynamic changes in chromosome methylation state to synchronize chromosome replication with cell-cycle regulation. We identified the N^6-methyladenine and 5-methylcytosine methylation state of every base pair at five times in the cell cycle to show that 4,515 GANTC sites, recognized by the CcrM methyltransferase, change from full- to hemimethylation upon passage of the replication fork. Significantly, 27 of the GANTC sites are protected from methylation at all times. We also identified (pp. E4658–E4667) four previously unknown methylation motifs and the cognate methyltransferase for two of these motifs. The ability to track the state of the methylome in exquisite temporal detail will be invaluable to investigations of microbial epigenetic regulation.

Dynamic faces speed up the onset of auditory cortical spiking responses during vocal detection

Chandramouli Chandrasekaran, Luis Lemus, and Asif A. Ghazanfar

We combine facial motion with voices to help us hear better, but the role that low-level sensory areas such as the auditory cortex may play in this process is unclear. We combined a vocalization detection task with auditory cortical physiology in monkeys to bridge this epistemic gap. Surprisingly, and contrary to previous assumptions and hypotheses, changes in firing rate had no clear relationship to the detection advantage that dynamic faces provided when listening for vocalizations (pp. E4668–E4677). Instead, dynamic faces uniformly sped up the onset of spiking activity in the auditory cortex, and this faster onset partially explains the behavioral benefits of combining faces and voices.

Rapid stimulus-evoked astrocyte Ca^{2+} elevations and hemodynamic responses in mouse somatosensory cortex in vivo

Barbara Lykke Lind, Alexey R. Brazhe, Sanne Barsballe Jessen, Florence C. C. Tan, and Martin J. Lauritzen

The morphology of astrocytes places them as likely contributors to communication between nerve cells and blood vessels. They are reported to respond with few and slow Ca^{2+} elevations, which exclude them as possible participants in initiation of blood flow responses or synapse communication. We establish (pp. E4678–E4687) that astrocytes have fast responses in addition to the slow. These rapid, brief Ca^{2+} responses were present in a large proportion of astrocytes. We were able to observe these changes due to a signal enhancement analysis, which is useful when responses are small compared with baseline activity. Our findings indicate a higher sensitivity than generally believed of astrocytes.

Arabidopsis thaliana AHL family modulates hypocotyl growth redundantly by interacting with each other via the PPC/DUF296 domain

Jianfei Zhao, David S. Favero, Hao Peng, and Michael M. Neff

Members of the *AT-HOOK MOTIF CONTAINING NUCLEAR LOCALIZED* (*AHL*) family are involved in various plant biological processes. Our findings (pp. E4688–E4697) reveal a molecular model whereby the AHLs interact with each other via the plant and prokaryote conserved (PPC)/domain of unknown function #296 (DUF296) domain to form homo-/hetero-complexes, possibly trimers. The AHL complex also interacts with other nuclear proteins to form a macromolecular complex that modulates plant growth and development. The coordinated action of AHLs requires an AT-hook motif capable of binding AT-rich DNA, as well as a PPC/DUF296 domain containing a conserved six-amino-acid region. Our proposed model provides a better understanding of the roles of *AHL* genes in regulating plant growth and development, which may in turn lead to better seedling establishment and increased yield.