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PNAS Plus Significance Statements

Gold surfaces and nanoparticles are protected by Au(0)–thiyl species and are destroyed when Au(I)–thiolates form

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Synthetic design strategies for gold surface protection and nanoparticle formation require knowledge of how protectant ligands bind. Sulfur compounds may protect gold surfaces using a weakly bound (physisorbed) form or a strongly bound (chemisorbed) one often assumed to be Au(I)–thiolate. However, chemical reaction conditions optimized for Au(I)–thiolate protection instead etch surfaces to produce molecular thin films. All experimental and calculated evidence indicates that chemisorbed surface species are actually bound mainly by strong van der Waals (aurophilic-like) forces. This understanding unifies gold–sulfur surface chemistry with that of all other ligands and also with that of gold compounds, forming the basis for future methodological developments. It is applied to predict intermediate species during the Brust–Schiffrin nanoparticle synthesis that are subsequently observed spectroscopically. (See pp. E1424–E1433.)

Temperature-driven global sea-level variability in the Common Era

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We present the first, to our knowledge, estimate of global sea-level (GSL) change over the last ∼3,000 years that is based upon statistical synthesis of a global database of regional sea-level reconstructions. GSL varied by ∼±8 cm over the pre-Industrial Common Era, with a notable decline over 1000–1400 CE coinciding with ∼0.2 °C of global cooling. The 20th century rise was extremely likely faster than during any of the 27 previous centuries. Semiempirical modeling indicates that, without global warming, GSL in the 20th century very likely would have risen by between −3 cm and +7 cm, rather than the ∼14 cm observed. Semiempirical 21st century projections largely reconcile differences between Intergovernmental Panel on Climate Change projections and semiempirical models. (See pp. E1434–E1441.)

Functional and topological diversity of LOV domain photoreceptors

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Photoreceptor proteins dynamically control many critical physiological processes in response to light across the whole phylogenetic order, including the regulation of circadian rhythms and photosynthesis. We created a comprehensive catalog of the protein architectures and biochemical functions of a ubiquitous class of natural photoreceptors, the light–oxygen–voltage sensitive (LOV) class of flavoproteins, including >4,000 new candidate LOVs, which nearly triples the sequence diversity known to date. Establishing the functional and structural diversity of LOVs will (*i*) shed light on how organisms adapt to environmental changes, (*ii*) elucidate the structure–function principles by which common photosensory inputs are transmitted into a multitude of cell signaling events, and (*iii*) beget novel "optogenetic" tools for light-driven physiological perturbation of cells expressing natural or engineered photoreceptors. (See pp. E1442–E1451.)

Novel genomic island modifies DNA with 7-deazaguanine derivatives

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The discovery of a novel modification system that inserts 7-deazaguanine derivatives in DNA, modifications thought until now to occur only in RNA, is an excellent illustration of the power of biological evolution to alter the ultimate function not only of the distinct proteins but also of entire metabolic pathways. The extensive lateral transfer of the gene cluster responsible for this modification highlights its significance as a previously unrecognized foreign DNA defense system that bacteria and phages use to protect their genomes. The characterization of these DNA modification pathways also opens the door to novel tools to manipulate nucleic acids. (See pp. E1452–E1459.)

Inactivation of 3-*hydroxybutyrate dehydrogenase* 2 delays zebrafish erythroid maturation by conferring premature mitophagy

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In addition to their well-documented roles in energy metabolism and heme biogenesis, mitochondria also play an important role in intracellular signaling, which is important for tissue and organ development. We previously showed that a cytosolic siderophore facilitates mitochondrial iron import in eukaryotes. Depletion of the siderophore by inactivating 3-hydroxy butyrate dehydrogenase 2 (*bdh2*), whose gene product is necessary for siderophore biogenesis, results in heme deficiency and delays erythroid maturation in developing zebrafish embryo. Here we show that inactivation of *bdh2* results in mitochondrial dysfunction, triggers their degradation by mitophagy, and hampers erythroid maturation. Reestablishment of *bdh2* restores mitochondrial function, prevents premature mitochondrial degradation, and promotes erythroid maturation. Our results demonstrate that mitochondrial communication with the nucleus is critical for erythroid development. (See pp. E1460–E1469.)

Biophysical principles predict fitness landscapes of drug resistance

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Development of predictive models of antibiotic resistance is challenging due to a lack of understanding of the relationship between molecular and fitness effects of mutations (the genotype–phenotype gap). Here we close the genotype–phenotype gap for an essential enzyme, dihydrofolate reductase (DHFR), which is an important target of the common antibiotic trimethoprim. We show that IC_{50} of trimethoprim resistance of *Escherichia coli* can be predicted, with high accuracy, from a unique combination of molecular properties of stepwise-resistant DHFR variants. These results show that the challenge to predict de novo evolutionary dynamics of antibiotic resistance lies in the need for accurate prediction of the effects of mutations on the molecular properties of target enzymes. (See pp. E1470–E1478.)

Membrane-bound MinDE complex acts as a toggle switch that drives Min oscillation coupled to cytoplasmic depletion of MinD

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The Min system of *Escherichia coli* uses the proteins MinD and MinE to form a standing wave oscillator on the membrane that prevents cell division at the cell poles. Using purified MinD and MinE, several dynamic patterns have been previously reconstituted on lipid bilayers. However, these dissimilar patterns occur under different reaction settings; therefore, the underlying mechanistic principles are unclear. By using a limiting supply of MinD, we reproduced standing wave oscillation on a flat bilayer. We find that periodic depletion of active MinD from solution is essential for the standing wave. Also, the MinD-to-MinE ratio on the bilayer acts as a toggle switch between membrane-binding and -release by MinD, which drives the oscillation. (See pp. E1479–E1488.)

Paracrine Wnt/β-catenin signaling mediates proliferation of undifferentiated spermatogonia in the adult mouse testis

Hinako M. Takase and Roeland Nusse

Spermatogonial stem cells are unique among adult tissue stem cells in their role in transmitting genetic information to the next generation. Germ-line stem cells in *Caenorhabditis elegans* and *Drosophila* are well studied because of their relatively simple organization with a clear anatomical niche, but the regulatory mechanisms behind mammalian spermatogonial stem cells are less well understood. In this report, we demonstrate that the proliferation of undifferentiated spermatogonia, including spermatogonial stem cells, is controlled by Wnt/β-catenin signaling. Wnts are secreted by Sertoli cells, which thereby act as a niche. To our knowledge, this work proves, for the first time, that Wnt/β-catenin signaling is involved in spermatogonial stem/progenitor cell regulation in vivo, and also uncovers its mode of action. (See pp. E1489–E1497.)

Axin2 marks quiescent hair follicle bulge stem cells that are maintained by autocrine Wnt/β-catenin signaling

Xinhong Lim, Si Hui Tan, Ka Lou Yu, Sophia Beng Hui Lim, and Roeland Nusse

Hair follicle stem cells (HFSCs) remain quiescent for long periods of time during the resting phase of the hair cycle. How they maintain their stemness and identity during quiescence while being responsive to growth-inducing cues remains poorly understood. Here, we identify *Axin2* as a previously unidentified marker of HFSCs and use it to show that quiescent HFSCs undergo and require active Wnt/β-catenin signaling. By mapping Wnt and its inhibitors with high sensitivity, we show that HFSCs secrete their own self-renewing Wnt signals and inhibitors that promote differentiation outside of the stem cell compartment. Our findings suggest that careful modulation of Wnt signaling may be important for the derivation and maintenance of HFSCs for alopecia treatment and drug screens. (See pp. E1498–E1505.)

FOXC1 maintains the hair follicle stem cell niche and governs stem cell quiescence to preserve long-term tissue-regenerating potential

Kenneth Lay, Tsutomu Kume, and Elaine Fuchs

Stem cells (SCs) of the hair follicle (HF) undergo cyclical bouts of activity during which hair regeneration occurs. They reside in a specialized niche, the bulge, which confers upon them extended periods of quiescence. Here, we identify Forkhead box C1 (FOXC1) as a key transcriptional regulator of HFSC activity and bulge maintenance. Loss of FOXC1 reduces the threshold for HFSC activation, causing excessive HFSC usage and dramatically shortening periods between hair growth cycles. Additionally, signs of weakened cellular junctions are seen within the niche, resulting in mechanically induced, premature loss of established hairs along with some SCs. The consequences of these defects are dire for aging animals, which display diminished HFSC niches and a sparse hair coat. (See pp. E1506–E1515.)

Insights into global diatom distribution and diversity in the world's ocean

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Diatoms, considered one of the most diverse and ecologically important phytoplanktonic groups, contribute around 20% of global primary productivity. They are particularly abundant in nutrient-rich coastal ecosystems and at high latitudes. Here, we have explored the dataset generated by *Tara* Oceans from a wide range of oceanic regions to characterize diatom diversity patterns on a global scale. We confirm the dominance of diatoms as a major photosynthetic group and identify the most widespread and diverse genera. We also provide a new estimate of marine planktonic diatom diversity and a global view of their distribution in the world's ocean. (See pp. E1516–E1525.)

Functions for diverse metabolic activities in heterochromatin

Xue Bessie Su and Lorraine Pillus

Eukaryotic genomes have distinct heterochromatic regions that silence gene transcription and other DNA transactions. In yeast, these regions are controlled by activities of the silent information regulator (SIR) complex, which are influenced by NAD^+ metabolism. We report results, starting from an in silico screen, that proteins functioning in a diverse range of amino acid metabolic activities influence chromatin silencing, thus expanding knowledge of connections between metabolism and epigenetic processes. In a case study, we found that glutamate dehydrogenase 1 (Gdh1) has two chromatin-based functions: controlling recruitment of the SIR complex and negatively regulating proteolysis of a conserved core histone to modulate gene expression. Mechanistically, Gdh1 contributes to silencing through α-ketoglutarate consumption, and high levels of α-ketoglutarate are shown to be detrimental to telomeric silencing. (See pp. E1526–E1535.)

A *PERIOD3* variant causes a circadian phenotype and is associated with a seasonal mood trait

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It has long been thought that sleep and mood are intimately connected in humans, but at present there are no known molecular links. We identified rare variants in the *PERIOD3* gene in persons with both altered sleep behavior and features of seasonal affective disorder. We show that these variants recapitulate circadian and mood phenotypes in mouse models. Although we were not able to test mood in fruit flies, we did uncover a sleep trait similar to that seen in humans in flies carrying the human variants. Our molecular studies reveal that the variants led to less stable PER3 protein and reduced the stabilizing effect of PER3 on PER1/PER2, providing a mechanistic explanation for the circadian trait. (See pp. E1536–E1544.)

Functional requirements of AID's higher order structures and their interaction with RNA-binding proteins

Samiran Mondal, Nasim A. Begum, Wenjun Hu, and Tasuku Honjo

This paper demonstrates that activation-induced cytidine deaminase (AID), an essential enzyme in antigen-induced antibody diversification, forms distinct ribonucleoprotein complexes depending on its structural states: namely monomers or dimers. The identified RNA-binding proteins are required for the function of AID: namely DNA cleavage or recombination. In addition, the complex formation between AID and heterogeneous nuclear ribonucleoproteins (hnRNPs) is RNA-dependent. (See pp. E1545–E1554.)

Immunoproteasome deficiency is a feature of non-small cell lung cancer with a mesenchymal phenotype and is associated with a poor outcome

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The success rate of therapeutic trials that target tumor antigens is quite limited. We demonstrate for the first time to our knowledge that lung cancer cells that have undergone epithelial-to-mesenchymal transition lose immunoproteasome expression, resulting in markedly reduced antigen presentation. Reduced expression of the immunoproteasome was associated with and can predict poor outcome in non-small cell lung carcinoma (NSCLC) patients. Induction of the immunoproteasome with IFNγ or 5-aza-2′-deoxycytidine (5-aza-dC) treatment can overcome this immune escape mechanism of mesenchymal cells by restoring functional HLA class I-bound peptides. These findings have substantial relevance for development of effective strategies to target tumor cells with inherent resistance to T cell-mediated immunotherapy. (See pp. E1555–E1564.)

Disruption of lipid homeostasis in the Gram-negative cell envelope activates a novel cell death pathway

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The cell envelope of Gram-negative bacteria contains two membranes and a cell wall located in the aqueous compartment between them. The outer membrane (OM) functions as a barrier that contributes to antibiotic resistance. We describe a dominant mutation in a gene for an OM lipoprotein that leads to cell death under starvation conditions in medium with limited cation concentrations. We show that death occurs not by rapid cell lysis but by a previously uncharacterized mechanism involving flow of material from the inner membrane to the OM that results in rupture of the inner membrane and the slow leakage of cytoplasmic contents. Our study highlights the vital need for balanced synthesis across the Gram-negative envelope and may empower the development of new therapeutics. (See pp. E1565–E1574.)

Excitatory synapses are stronger in the hippocampus of Rett syndrome mice due to altered synaptic trafficking of AMPA-type glutamate receptors

Wei Li, Xin Xu, and Lucas Pozzo-Miller

Rett syndrome is the most common intellectual disability in women after Down syndrome (1:10,000 incidence). Different mouse models of intellectual disability and autism exhibit deficits in synaptic plasticity, including impaired long-term potentiation (LTP) in mice lacking methyl-CpG-binding protein 2 (MeCP2); however, the bases of this deficit remain unclear. Using a combination of electrophysiology, time-lapse imaging, cell biology, and biochemistry, we provide direct evidence that

naïve hippocampal synapses in Rett mice have all the hallmarks of potentiated synapses. Rett synapses also fail to insert and remove AMPA receptors properly to activated synapses, which freezes them in a nonplastic state. Our findings provide molecular, cellular, and network mechanisms underlying enhanced excitatory synaptic transmission and impaired LTP in Rett mice, identifying previously unidentified molecular targets for therapeutic intervention. (See pp. E1575–E1584.)