PNAS Plus Significance Statements

Surveying the sequence diversity of model prebiotic peptides by mass spectrometry

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Peptides and proteins are essential for life as we know it, and likely played a critical role in the origins of life as well. In recent years, much progress has been made in understanding plausible routes from amino acids to peptides. However, little is known about the diversity of sequences that could have been produced by abiotic condensation reactions on the prebiotic earth. In this study, multidimensional separations were coupled with mass spectrometry to detect and sequence mixtures of model proto-peptides. It was observed that, starting with a few monomers, proto-peptide diversity increased rapidly following cycling. Experimental proto-peptide sequences were compared with theoretically random sequences, revealing a high sequence diversity of plausible monomer combinations. (See pp. E7652–E7659.)

Neutral high-generation phosphorus dendrimers inhibit macrophage-mediated inflammatory response in vitro and in vivo

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Inflammation is a relevant part of the physiological response of the immune system to fight infectious diseases. However, excessive inflammation can also participate in the pathogenesis of several diseases. To open new avenues to treat the negative aspects of inflammation, we have used biocompatible nanoparticles, neutrally charged G3-G4 phosphorus dendrimers, which are not toxic and have good solubility and chemical stability in aqueous solutions. These nanoparticles are very efficient, in vitro and in vivo, to tackle the inflammatory response produced by different agents. In addition, the high number of chemically modifiable terminal groups on the surface of these nanodevices opens the high possibility of incorporating into them specific therapeutic groups which provide multifunctional therapeutic abilities. (See pp. E7660–E7669.)

Amorphous calcium carbonate particles form coral skeletons

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Whether coral skeleton crystals grow by attachment of ions from solution or particles from tissue determines (*i*) corals' growth rate, (*ii*) how they survive acidifying oceans, and (*iii*) the isotopes in the crystals used for reconstructing ancient temperatures. Our data show that two amorphous precursors exist, one hydrated and one dehydrated amorphous calcium carbonate; that these are formed in the tissue as ∼400-nm particles; and that they attach to the surface of coral skeletons, remain amorphous for hours, and finally crystallize into aragonite. Since these particles are formed inside tissue, coral skeleton growth may be less susceptible to ocean acidification than previously assumed. Coral bleaching and postmortem dissolution of the skeleton will occur, but a calcification crisis may not. (See pp. E7670–E7678.)

tRNAs and proteins use the same import channel for translocation across the mitochondrial outer membrane of trypanosomes

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In most eukaryotes mitochondrial function requires not only import of proteins but also import of at least some tRNAs. Trypanosomes are extreme in that they lack mitochondrial tRNA genes, and therefore must import all of their mitochondrial tRNAs from the cytosol. Here we show that in trypanosomes both proteins and tRNAs use the same β-barrel protein pore to be translocated across the mitochondrial outer membrane. Moreover, we show that tRNA import can be uncoupled from protein import. Based on these results, we propose the "alternate import model," in which tRNAs use the same outer membrane import pore as proteins but are imported as naked molecules. The model combines features of the previously proposed "coimport" and "direct import" models. (See pp. E7679–E7687.)

Quantitative tests of a reconstitution model for RNA folding thermodynamics and kinetics

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We propose and test predictions of a thermodynamic and kinetic model for RNA tertiary folding that is based on separable energetic contributions of RNA elements. We define these contributions based on the principle features of RNA, and we test the basic predictions of separability by determining whether the energetic contributions of one component are affected by changes in another component. Our results support energetic separability of RNA elements and suggest that it may

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be possible to deconstruct RNAs into smaller parts that can be studied in isolation such that the individual folding behaviors of these parts can be used to "reconstitute" the folding of the original RNA. (See pp. E7688–E7696.)

Global metabolic reprogramming of colorectal cancer occurs at adenoma stage and is induced by MYC

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Metabolic reprogramming is one of the hallmarks of cancer. However, the underlying mechanisms that regulate cancer metabolism are poorly understood. Here we performed multiomics-based analysis of paired normal–tumor tissues from patients with colorectal cancer, which revealed that the protooncogene protein MYC regulated global metabolic reprogramming of colorectal cancer by modulating 215 metabolic reactions. Importantly, this metabolic reprogramming occurred in a manner not associated with specific gene mutations in colorectal carcinogenesis. For many years, small-molecule or biologic inhibitors of MYC have been required. Here we demonstrate that knockdown of MYC downstream pyrimidine synthesis genes contributes to the suppression of colorectal cancer cell proliferation similar to MYC, and thus pyrimidine synthesis pathways could be potential targets for colorectal cancer therapy. (See pp. E7697–E7706.)

TFG facilitates outer coat disassembly on COPII transport carriers to promote tethering and fusion with ER–Golgi intermediate compartments

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The endoplasmic reticulum (ER) serves as a platform for the packaging of most secretory proteins into conserved coat protein complex II (COPII)-coated transport carriers destined for ER–Golgi intermediate compartments (ERGIC) in animal cells. In this work, we demonstrate that Trk-fused gene (TFG), a protein implicated in multiple neurodegenerative diseases and oncogenesis, functions in this pathway by interacting directly with the COPII protein Sec23. Specifically, we show that TFG outcompetes interactions between the inner and outer layers of the COPII coat, indicating that TFG promotes the uncoating process after transport carriers undergo scission from the ER. Moreover, we demonstrate that TFG simultaneously captures and concentrates COPII transport carriers at the ER/ERGIC interface to enable the rapid movement of secretory cargoes to the ERGIC. (See pp. E7707–E7716.)

Clipping of arginine-methylated histone tails by JMJD5 and JMJD7

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Enzymes responsible for the clipping of histone tails and removal of arginine-methylated histone tails still remain elusive. The underlying mechanism of high histone turnover rate in nonproliferated cells is still a mystery. How RNA polymerase II overcomes nucleosome barriers during transcription is unknown. This article described the discovery of a JmjC domain containing subfamily members JMJD5 and JMJD7, which could be responsible for these unsolved puzzles in epigenetics and transcription fields. (See pp. E7717–E7726.)

Shear force-based genetic screen reveals negative regulators of cell adhesion and protrusive activity

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We report a forward genetic screen to identify genes involved in cell adhesion and motility. Cells with mutations in these genes have increased adhesion, flattened morphology, and decreased motility. The mutants display increased cytoskeletal and signal transduction network activity suggesting that these genes are negative regulators. The GFP-tagged localization of these proteins shows the remarkable diversity in the regulation of these cell behaviors. Several of the identified proteins have strong homologs throughout metazoans and have relevance to human disease. Because many of the resulting mutant phenotypes are similar to those of cells lacking PTEN or expressing active Ras GTPases, these gene families are promising cancer targets in humans. Better understanding of these pathways holds the possibility for therapeutic intervention. (See pp. E7727–E7736.)

Early photosynthetic eukaryotes inhabited low-salinity habitats

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Although it is widely accepted that the chrloroplasts in photosynthetic eukaryotes can be traced back to a single cyanobacterial ancestor, the nature of that ancestor remains debated. Chloroplasts have been proposed to derive from either early- or late-branching cyanobacterial lineages, and similarly, the timing and ecological setting of this event remain uncertain. Phylogenomic and Bayesian relaxed molecular clock analyses show that the chloroplast lineage branched deep within the cyanobacterial tree of life ~2.1 billion y ago, and ancestral trait reconstruction places this event in low-salinity environments. The chloroplast took another 200 My to become established, with most extant groups originating much later. Our analyses help to illuminate the little known evolutionary history of early life on land. (See pp. E7737–E7745.)

Human genetic variation in *VAC14* regulates *Salmonella* invasion and typhoid fever through modulation of cholesterol

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Salmonella enterica serovar Typhi (*S*. Typhi) causes ~20 million cases of typhoid fever every year. We carried out a genome-wide association study to identify genetic differences that correlate with the susceptibility of cells from hundreds of individuals to *S*. Typhi invasion. A SNP in *VAC14* was associated with susceptibility to *S*. Typhi invasion and *VAC14* expression. Cells mutated for *VAC14* displayed increased *S*. Typhi docking due to increased plasma membrane cholesterol levels. The same SNP was associated with risk of typhoid fever in a Vietnamese population. Furthermore, treating zebrafish with a cholesterol-lowering drug reduced their susceptibility to *S.* Typhi infection. Therefore, this work demonstrates the power of coupling multiple genetic association studies with mechanistic dissection for understanding infectious disease susceptibility. (See pp. E7746–E7755.)

A-to-I RNA editing is developmentally regulated and generally adaptive for sexual reproduction in *Neurospora crassa*

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This study systematically identified adenosine to inosine (A-to-I) editing sites in *Neurospora crassa* and showed the existence of stage-specific editing events at different sexual stages. Unlike in humans, fungal A-to-I editing mainly occurred in coding regions and caused nonsynonymous changes that significantly increased proteome complexity. In general, nonsynonymous editing sites in *Neurospora* are adaptive and favored by positive selection. RNA editing enables stage-specific functions or expression of proteins important for different sexual developmental processes. Some editing events are well conserved and may affect genes important for other genetic and epigenetic phenomena occurring during sexual reproduction. Overall, our results provide insights into the complex regulation of sexual development and reveal the role of A-to-I editing for adaptive evolution in *Neurospora*. (See pp. E7756–E7765.)

NLRP3 mutation and cochlear autoinflammation cause syndromic and nonsyndromic hearing loss DFNA34 responsive to anakinra therapy

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This study identifies a mutation in the *NLRP3* gene that causes sensorineural hearing loss in human patients. *NLRP3* encodes a protein important for innate immunity, secretion of the potent cytokine IL-1β, and inflammation. The hearing loss in three affected members of one family improved or completely resolved after treatment with IL-1β blockade therapy. This study shows that the mouse *Nlrp3* gene is expressed in immune macrophage-like cells throughout the inner ear, which can be activated to release the potent cytokine IL-1β. These observations suggest that mutations of *NLRP3* may cause hearing loss by local autoinflammation within the inner ear. This mechanism could underlie a variety of hearing-loss disorders of unknown etiology that might respond to IL-1β blockade therapy. (See pp. E7766–E7775.)

Imaging the emergence and natural progression of spontaneous autoimmune diabetes

James F. Mohan, Rainer H. Kohler, Jonathan A. Hill, Ralph Weissleder, Diane Mathis, and Christophe Benoist

Dynamics and interactions of immunocytes infiltrating the pancreas during the natural progression of autoimmune diabetes are largely unknown. The construction of diabetes-prone nonobese diabetic mice with a panel of fluorescent reporters that illume infiltrating cells of the innate and adaptive immune systems, combined with intravital imaging of the pancreas, provide novel perspectives on the autoimmune process and on the ballet between aggressive and regulatory cells. (See pp. E7776–E7785.)

Integrative single-cell and cell-free plasma RNA transcriptomics elucidates placental cellular dynamics

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The human placenta is a dynamic and cellular heterogeneous organ, which is critical in fetomaternal homeostasis and the development of preeclampsia. Previous work has shown that placenta-derived cell-free RNA increases during pregnancy. We applied large-scale microfluidic single-cell transcriptomic technology to comprehensively characterize cellular heterogeneity of the human placentas and identified multiple placental cell-type–specific gene signatures. Analysis of the cellular signature expression in maternal plasma enabled noninvasive delineation of the cellular dynamics of the placenta during pregnancy and the elucidation of extravillous trophoblastic dysfunction in early preeclampsia. (See pp. E7786–E7795.)

Investment in secreted enzymes during nutrient-limited growth is utility dependent

Brent Cezairliyan and Frederick M. Ausubel

Bacteria secrete enzymes into the environment to digest macromolecules into smaller molecules that can be used as nutrients for growth. Secreted enzymes have potential benefits but also entail costs in the form of biomass and energy. How do bacteria determine how much of them to make? Using a system in which nutrient acquisition requires production of secreted enzymes, we infer that bacteria produce secreted signals in proportion to the benefit they would receive from the action of secreted enzymes. Investment in secreted enzymes is adjusted according to the magnitude of those signals. Our model provides a framework that can be applied to bacterial growth in many environments from contaminated food to microbial communities within a host. (See pp. E7796–E7802.)

Differential HspBP1 expression accounts for the greater vulnerability of neurons than astrocytes to misfolded proteins

Ting Zhao, Yan Hong, Peng Yin, Shihua Li, and Xiao-Jiang Li

It remains unclear why astrocytes are affected to a lesser extent than neurons in a variety of neurodegenerative diseases. We report the higher activity of C terminus of Hsp70-interacting protein (CHIP), cochaperone of Hsp70, in astrocytes than in neurons, which not only promotes the degradation of misfolded proteins, but also upregulates levels of basal and stress-induced Hsp70 in astrocytes. Furthermore, the low activity of CHIP in neurons is caused by the abundant expression of HspBP1, an inhibitor of CHIP. Knocking down HspBP1 in neurons prevents the accumulation and aggregation of the Huntington's disease (HD) protein and ameliorates neuropathology in a HD knockin mouse model. These findings suggest that HspBP1 accounts for differential vulnerabilities of neurons and glia to misfolded proteins. (See pp. E7803–E7811.)

5-hydroxymethylcytosine accumulation in postmitotic neurons results in functional demethylation of expressed genes

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The main insight from this study is that the role of 5-hydroxymethylcytosine (5hmC) in postmitotic neurons is to sculpt the genome occupancy of the very abundant 5-methylcyctosine binding protein 2 (MeCP2). Accumulation of 5hmCG in transcribed genes replaces high-affinity 5mCG binding sites with low-affinity sites, decreasing MeCP2 occupancy over the transcription unit and removing its repressive effect. We refer to this role for 5hmCG as "functional demethylation" because its biochemical effect with respect to MeCP2 is equivalent to chemical demethylation: Loss of high-affinity sites for interaction in the genome. This concept reinforces the roles of 5hmC in demethylation in dividing cells by a mechanism that achieves the same goal without requiring cell division or DNA damage. (See pp. E7812–E7821.)

Mechanisms of ovipositor insertion and steering of a parasitic wasp

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Using slender probes to drill through solids is challenging, but desirable, due to minimal disturbances of the substrate. Parasitic wasps drill into solid substrates and lay eggs in hosts hidden within using slender probes and are therefore a good model for studying mechanical challenges associated with this process. We show that wasps are able to probe in any direction with respect to their body orientation and use two methods of insertion. One of the methods implies a minimal net pushing force during drilling. Steering was achieved by adjusting the asymmetry of the probe's distal end. Knowledge on probing mechanisms of wasps is important for the understanding of the hymenopteran evolution and for the development of minimally invasive steerable probes. (See pp. E7822–E7831.)

Reactive oxygen species extend insect life span using components of the insulin-signaling pathway

Xiao-Shuai Zhang, Tao Wang, Xian-Wu Lin, David L. Denlinger, and Wei-Hua Xu

Oxidative damage is frequently associated with aging and aging-related disease, but, paradoxically, several recent studies have shown that artificial boosts of reactive oxygen species (ROS) can also extend life span in young individuals. Here, we show that physiological levels of ROS promote diapause, thereby extending life span in pupae of the moth *Helicoverpa armigera*. Insect diapause, like the dauer stage of nematodes, is a period of developmental rest that results in a profound extension of life span. ROS appears to contribute to this life span extension by acting through components of the insulin-signaling pathway. Our results thus suggest a new molecular mechanism regulating life span and help to explain the dual nature of ROS action in animals. (See pp. E7832–E7840.)

Exploring regulation in tissues with eQTL networks

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A core tenet in genetics is that genotype influences phenotype. In an individual, the same genome can be expressed in substantially different ways, depending on the tissue. Expression quantitative trait locus (eQTL) analysis, which associates genetic variants at millions of locations across the genome with the expression levels of each gene, can provide insight into genetic regulation of phenotype. In each of 13 tissues we performed an eQTL analysis, represented significant associations as edges in a network, and explored the structure of those networks. We found clusters of eQTL linked to shared functions across tissues and tissue-specific clusters linked to tissue-specific functions, driven by genetic variants with tissue-specific regulatory potential. Our findings provide unique insight into the genotype–phenotype relationship. (See pp. E7841–E7850.)