PNAS Plus Significance Statements

On the origin of the elusive first intermediate of CO_2 electroreduction

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The understanding of a catalytic reaction starts with understanding its first elementary step. Surprisingly, despite the large number of studies, it is unclear whether one common or two different first intermediates control the selectivity of CO_2 electroreduction to formate and CO. We settle this controversy for Cu, which is best known for its unique capacity to synthesize C_{1+} products but is just emerging as a superior earth-abundant catalyst for CO and formate. We provide solid experimental and theoretical support of the one common first-intermediate (Hori's) model, the first intermediate being carboxylate. This outcome is an essential milestone toward accurate specification of the reaction descriptors in the growing effort to accelerate the discovery of a viable CO_2 electroreduction catalyst. (See pp. E9261–E9270.)

Assimilation of formic acid and CO_2 by engineered *Escherichia coli* equipped with reconstructed one-carbon assimilation pathways

Junho Bang and Sang Yup Lee

While biological utilization of one-carbon (C1) compounds has attracted much attention, previous studies have focused mainly on the utilization of CO_2. Here, we report development of *Escherichia coli* strains capable of assimilating formic acid (FA) and CO_2 through the C1 assimilation pathway, synthesizing pyruvate from FA and CO_2 by establishing the reconstructed tetrahydrofolate cycle and the reverse glycine cleavage pathway. To generate energy and redox while using less glucose, a heterologous formate dehydrogenase was introduced together with the C1 assimilation pathway. The resulting strain could utilize FA and CO_2 as sole carbon sources for sustaining growth. This work demonstrates that the combined use of the C1 assimilation pathway and formate dehydrogenase allows *E. coli* to utilize FA and CO_2 efficiently. (See pp. E9271–E9279.)

Effects of protein size, thermodynamic stability, and net charge on cotranslational folding on the ribosome

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There is increasing interest to understand how proteins fold as they are synthesized on the ribosome. However, we still lack basic knowledge such as how protein size, net charge, and thermodynamic stability impact cotranslational folding. Here, we have studied eight proteins of increasing size and provide a comprehensive picture of how the location in the ribosome exit tunnel where a protein folds correlates with protein size. Moreover, we demonstrate that the force exerted on the nascent chain by protein folding varies linearly with the thermodynamic stability of the folded state, and that the ribosome environment disfavors folding of domains of high net-negative charge. These findings establish important basic facts about cotranslational folding. (See pp. E9280–E9287.)

Synchronized mechanical oscillations at the cell–matrix interface in the formation of tensile tissue

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Cells in developing tissues tune the mechanical properties of their extracellular matrix in the face of changes in external loading to maintain a tension "set point," but the mechanism has not been studied. We show here that the set point is an equilibrium between cell-independent stress relaxation in the matrix and nonmuscle myosin II-dependent contraction of the cell. The matrix-dependent and cell-dependent phases exhibit an oscillating tension component. Detensioning, and also retensioning, induces synchronization of mechanical oscillations between neighboring cells. We propose that mechanical oscillation at the cell–matrix interface is a key mechanism in the ability of embryonic fibroblasts to sense their mechanical environment, especially the viscoelastic state of the relaxed tendon. (See pp. E9288–E9297.)

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Control of CCND1 ubiquitylation by the catalytic SAGA subunit USP22 is essential for cell cycle progression through G1 in cancer cells

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The deubiquitylase USP22 is frequently overexpressed in cancer and contributes to tumorigenesis by driving cell cycle progression. Current models define USP22 as functional mediator of gene regulation and chromatin modification, working within the SAGA transcriptional cofactor complex. Here we report a catalytic role for USP22 distinct from its well-characterized transcription regulatory capacity. USP22 directly stabilizes the essential G1-cyclin, CCND1, protecting it from proteasome-mediated degradation via deubiquitylation. Our findings reveal a pathway that regulates CCND1, while also raising the possibility that simultaneously targeting USP22 may allow the use of less toxic doses of the new wave of cancer therapies that target the cyclin/CDK complex. Finally, these results provide a mechanistic explanation for the effects of USP22 in cancer cell cycle control. (See pp. E9298–E9307.)

Screening for genes that regulate the differentiation of human megakaryocytic lineage cells

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Megakaryocyte progenitors (MkPs), derived from hematopoietic stem cells (HSCs), play major roles in hemostasis, thrombosis, inflammation, and vascular biology through generating platelets. However, the regulatory factors involved in MkP differentiation from HSCs are largely unknown. Here, we utilized a unique genomic approach, including the microarray gene expression commons platform, CRISPR/Cas9-mediated gene deletion, lentivirus-mediated gene overexpression, as well as multicolor flow cytometry and functional assays, and identified 10 genes that are highly expressed in MkPs and required for and can promote MkP generation from HSCs. In addition, we found inhibition of histone deacetylase activity increased MkP differentiation. Our results will not only shed light on the regulations of MkPs, but also facilitate efficient generation of MkPs and platelets for clinical applications. (See pp. E9308–E9316.)

Inhibition of cIAP1 as a strategy for targeting c-MYC–driven oncogenic activity

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Dysregulated expression of master transcription factor c-MYC has been shown to promote proliferation and cell survival programs in cancer cells to mediate resistance to anticancer therapies and promote metastasis. Development of pharmacological agents to inhibit c-MYC as an anticancer therapy is a longstanding but elusive goal in the cancer field. Our study provides a potential widely applicable pharmacological strategy for targeting c-MYC–driven oncogenic activity by inhibiting cIAP1 E3 ubiquitin ligase activity as a treatment for cancers. Furthermore, we demonstrate the pharmacological interference in the dynamic interaction of an E3 ubiquitin ligase with its E2s as a strategy for inhibiting ubiquitination reactions. (See pp. E9317–E9324.)

Targeted profiling of RNA translation reveals mTOR-4EBP1/2-independent translation regulation of mRNAs encoding ribosomal proteins

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The PI3K-Akt-mTOR pathway integrates signaling information from various mitogen and nutrient sensors to regulate cell growth and proliferation. Hyperactivation of this pathway has been observed in a number of diseases, including many cancers. Pharmacological inhibition of mechanistic target of rapamycin (mTOR) causes immediate suppression of cell-wide protein synthesis; interestingly, this effect is not uniform across all transcripts, but is biased toward mRNAs encoding ribosomal proteins. In this study, we developed a rapid, scalable, and targeted assay for RNA translation that enabled us to identify translation modulators of ribosomal proteins that act independent of mTOR signaling. These modulators include chemical compounds, acting via their "off-target" effects, as well as certain metabolic perturbations, and together, they represent a noncanonical mode of regulating ribosome biogenesis and protein synthesis capacity. (See pp. E9325–E9332.)

Physical basis for long-distance communication along meiotic chromosomes

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Formation of viable sex cells, such as eggs and sperm in humans, occurs during a special type of cell division (meiosis), in which parental chromosomes (homologs) must separate from each other. In most species this process requires a physical connection between homologs; these connections, called "cross-overs," arise from DNA breaks, which occur at high frequency at special sites called "hotspots." Successful meiosis requires that DNA double-strand breaks (DSBs) and the resulting cross-overs be carefully controlled. We describe here a physical mechanism for control of DSBs between distant hotspots via the 3D clustering of hotspots bound by their determinant proteins. Based on these results, we propose a physical mechanism for crossover interference, which was discovered over 100 y ago but whose mechanism has remained elusive. (See pp. E9333–E9342.)

Behavior of homing endonuclease gene drives targeting genes required for viability or female fertility with multiplexed guide RNAs

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Homing endonuclease gene (HEG)-based gene drive can bring about population suppression when genes required for viability or fertility are targeted. However, these strategies are vulnerable to failure through mechanisms that create alleles resistant to cleavage but that retain wild-type gene function. We show that resistance allele creation can be prevented through the use of guide RNAs designed to cleave a gene at four target sites. However, homing rates were modest, and the HEGs were unstable during homing. In addition, use of a promoter active in the female germline resulted in levels of HEG carryover that compromised the viability or fertility of HEG-bearing heterozygotes, thereby preventing drive. We propose strategies that can help to overcome these problems in next-generation HEG systems. (See pp. E9343–E9352.)

Utilizing TAPBPR to promote exogenous peptide loading onto cell surface MHC I molecules

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MHC class I molecules present small fragments of proteins from within the cell to alert the immune system to infection and cellular damage. Two protein accessory proteins, tapasin and TAPBPR, assist in the loading and selection of these peptides inside the cell. Here we show that one of these proteins, TAPBPR, surprisingly still works when delivered to the outside of cells and can be used to load peptides from viruses and tumours directly on surface MHC molecules. Therefore, we have found an efficient way to override the peptides naturally presented by cells and can use this to target immune responses against cells. This may prove beneficial to mount immune responses against cancer in the future. (See pp. E9353–E9361.)

IL-33 promotes recovery from acute colitis by inducing miR-320 to stimulate epithelial restitution and repair

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We clarify that the normal, inherent function of IL-33 following acute, resolving colitis is protection, inducing proliferation and restitution of ST2L-bearing intestinal epithelial cells (IECs). Importantly, this response occurs in otherwise healthy, immunocompetent C57BL/6J (B6) mice and may be different in other models possessing genetic and/or immunologic abnormalities that predispose to colitis, similar to patients with inflammatory bowel disease. Mechanistically, although the molecular processes responsible for control of microRNA (miR) biogenesis in response to challenge remain largely unknown, we report that IL-33 augments epithelial miR-320, which increases IEC proliferation and wound closure that is significantly diminished upon specific miR-320 inhibition. This study provides the rationale for the potential therapeutic use of either IL-33 or miR-320A to obtain optimal gut mucosal healing and the resolution of inflammation. (See pp. E9362–E9370.)

Chloride regulates dynamic NLRP3-dependent ASC oligomerization and inflammasome priming

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The NLRP3 inflammasome is a multimolecular protein complex which is responsible for regulating the processing and secretion of the proinflammatory cytokine interleukin-1β. Understanding the mechanisms regulating the assembly of the inflammasome is of outstanding biological interest and importance, not least because of the contributions of the NLRP3 inflammasome to disease processes. Here we report insights into the dynamic nature of inflammasome oligomerization and its dependence on chloride ions. These studies reveal an additional layer of regulation and priming that can further enhance and drive inflammatory responses. (See pp. E9371–E9380.)

Substance P and IL-33 administered together stimulate a marked secretion of IL-1β from human mast cells, inhibited by methoxyluteolin

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Mast cells are mandatory for allergic reactions and participate in inflammatory responses in which the peptide substance P (SP) and the cytokine IL-33 are involved. This report shows that SP administered together with IL-33 to cultured human mast cells causes a marked increase in the secretion and gene expression of IL-1β. These responses are mediated via the activation of the SP receptor NK-1 and the IL-33 receptor ST2 and can be inhibited by the natural flavonoid methoxyluteolin. These findings highlight the important role of SP and IL-33 in mast cell secretion of IL-1β and point to targets for the development of therapies for inflammatory diseases. (See pp. E9381–E9390.)

Developmental stage-specific proliferation and retinoblastoma genesis in RB-deficient human but not mouse cone precursors

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Retinoblastoma is a childhood tumor that forms in response to mutations in the *RB1* gene and loss of functional RB protein. Prior studies suggested that retinoblastomas arise from cone photoreceptor precursors, whereas mouse models yield tumors deriving from other retinal cell types and lacking human retinoblastoma features. We show that in cultured human retinae, retinoblastomas initiate from RB-depleted cone precursors that are in a specific maturation state and form premalignant retinomas prior to retinoblastoma lesions, as is believed to occur in retinoblastoma patients. In contrast, Rb-deficient mouse cone precursors of similar maturation state and supplemented with human cone precursor-specific oncoproteins fail to proliferate. Thus, human species-specific developmental features underlie retinoblastoma genesis and may challenge the production of accurate retinoblastoma models. (See pp. E9391–E9400.)

High-resolution structures of HIV-1 Gag cleavage mutants determine structural switch for virus maturation

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The main structural component of HIV-1 is the Gag polyprotein. During virus release, Gag is cleaved by the viral protease at five sites, triggering a major change in the structure and morphology of the virus. This transition, called maturation, is required to make an infectious virion. We used cryoelectron tomography to obtain high-resolution structures of Gag inside virus particles carrying mutations that block specific combinations of cleavage sites. Analysis of these structures suggests that different combinations of cleavages can destabilize a bundle of alpha-helices at the C terminus of CA. This destabilization, rather than formation of a beta-hairpin at the N terminus of CA as previously suggested, acts as the structural switch for maturation of the virus into its infectious form. (See pp. E9401–E9410.)

The PqsE and RhlR proteins are an autoinducer synthase–receptor pair that control virulence and biofilm development in *Pseudomonas aeruginosa*

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The human pathogen *Pseudomonas aeruginosa* is the leading cause of hospital-acquired infections and, moreover, is resistant to commonly used antibiotics. *P. aeruginosa* uses the cell-to-cell communication process called quorum sensing (QS) to control virulence. QS relies on production and response to extracellular signaling molecules called autoinducers. Here, we identify the PqsE enzyme as the synthase of an autoinducer that activates the

QS receptor RhlR. We show that the PqsE-derived autoinducer is the key molecule driving *P. aeruginosa* biofilm formation and virulence in animal models of infection. We propose that PqsE and RhlR constitute a QS synthase–receptor pair, and that this system can be targeted for antimicrobial development. (See pp. E9411–E9418.)

Changes in membrane properties of rat deep cerebellar nuclear projection neurons during acquisition of eyeblink conditioning

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Although large ensembles of neurons have been found to change as a function of learning and memory, localizing those changes to the individual neurons directly involved in a specific task has been challenging. Using whole-cell recording of deep cerebellar nuclear neurons (DCN) and a transsynaptic viral tracer, we found motor learning induced significant changes in membrane properties of rat DCN projection neurons including a reduced after-hyperpolarization amplitude and shortened latency for both evoked DCN action potentials and rebound spikes. These learning-specific changes in DCN excitability have not previously been reported in any species or task. (See pp. E9419–E9428.)

Contributions of the glycocalyx, endothelium, and extravascular compartment to the blood–brain barrier

Nikolay Kutuzov, Henrik Flyvbjerg, and Martin Lauritzen

The vascular endothelium constitutes the main barrier that restricts the transport of molecules from blood to brain. However, the barrier properties of structures adjacent to the vascular endothelium are understudied. Based on two-photon microscopy imaging of single cortical capillaries, we found that the blood–brain barrier (BBB) consisted of at least three elements: the endothelial glycocalyx, which forms a barrier on the blood side to large but not small molecules; the endothelium; and the basement membrane and astrocyte endfeet—the final line of defense on the brain side. All three elements restricted permeation of large molecules and should be taken into account when studying drug delivery and disease states. (See pp. E9429–E9438.)

Phasic locus coeruleus activity regulates cortical encoding of salience information

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Locus coeruleus (LC) function has been associated with focused attention across species. LC neurons fire tonically or with short phasic bursts. We found that phasic, but not tonic, LC activity produced attentional signals across cortex, including the P300 event-related potential, and revealed distinct long-latency signals in sensory networks. These long-latency signals were seen in subpopulations normally activated by intense or salient stimuli, meaning that phasic LC activation produced salience in sensory neurons. Phasic LC-generated sensory salience signals were tightly temporally regulated, and precisely timed phasic LC activation was able to generate "false salience" in sensory processing networks, in the absence of intense stimuli. Importantly, LC-induced salience signals occurred without changes in arousal, demonstrating independent mechanisms of LC-mediated arousal and attention. (See pp. E9439–E9448.)

cTAGE5/MEA6 plays a critical role in neuronal cellular components trafficking and brain development

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Neural development is essential for the formation of neuronal networks and brain function. cTAGE5/MEA6 plays a critical role in the secretion of proteins, including very low density lipoprotein and insulin; however, its role in the transport of cellular (nonsecretory) components and in brain development has not been previously explored. Here, we show that cTAGE5/MEA6 is essential for neural development, and knockout of cTAGE5/MEA6 in the mouse brain leads to severe neural developmental defects. The underlying mechanisms for the role of cTAGE5/MEA6 in brain development have been explored in this study. Interestingly, mutations in cTAGE5/MEA6 have been found in patients with Fahr's disease. Thus, our study also provides insight into the possible pathogenesis mechanisms of this neurological disorder. (See pp. E9449–E9458.)

Leak potassium channels regulate sleep duration

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To address molecular mechanisms regulating sleep duration, a simple computational model of a cortical neuron [simplified averaged neuron (SAN) model], which recapitulates the electrophysiological characteristics of slow-wave sleep (SWS) and wakefulness, is developed in this study. Comprehensive bifurcation and detailed mathematical analyses predicted that leak K^+ channels play a role in generating the cortical electrophysiological characteristics of SWS, leading to a hypothesis that leak K^+ channels play a role in the regulation of sleep duration. We comprehensively generated and analyzed 14 knockout mice of the leak K^+ channel family, which demonstrated that impairment of the leak K^+ channel (*Kcnk9*) decreases sleep duration. The results confirm the validity of the SAN model and suggest a molecular mechanism regulating sleep duration. (See pp. E9459–E9468.)

Ultradian calcium rhythms in the paraventricular nucleus and subparaventricular zone in the hypothalamus

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Despite that the various functions in mammals fluctuate in the ultradian fashion, the origin and mechanism of the rhythm are largely unknown. In this study, we found synchronous ultradian calcium rhythms in the hypothalamic paraventricular nucleus (PVN), subparaventricular zone (SPZ), and suprachiasmatic nucleus (SCN). The ultradian rhythms were originated from the SPZ-PVN region and transmitted to the SCN. Neurochemical interventions revealed that the glutamatergic mechanism is critical for generation and a tetrodotoxin-sensitive neural network for synchrony of the ultradian rhythm. The GABAergic system could have a role in refining the circadian output signals. The study provides the first clue to

understand the loci and mechanism of ultradian rhythm in the hypothalamus. (See pp. E9469–E9478.)

GIRK currents in VTA dopamine neurons control the sensitivity of mice to cocaine-induced locomotor sensitization

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Activation of G protein-gated inwardly rectifying potassium (GIRK) channels inhibits neuronal activity in the brain, but details are lacking on how this important pathway influences neural circuits in the reward pathway. Here, we provide an example of where control of trafficking of GIRK channels by a cytoplasmic protein, sorting nexin 27, determines the sensitivity of mice to cocaine in a model of addiction known as locomotor sensitization. These results implicate GIRK channels as a therapeutic target for treating addiction, as well as other psychiatric disorders involving dopamine dysregulation. (See pp. E9479–E9488.)

Uneven balance of power between hypothalamic peptidergic neurons in the control of feeding

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The interplay between the anorexigenic and orexigenic neurons in the arcuate nucleus that contributes to the control of feeding remains elusive. Using optogenetic stimulation, we show that activation of POMC neurons rapidly inhibits feeding behavior in fasted animals. However, simultaneous stimulation of both POMC neurons and a subset of the orexigenic neurons that express AgRP is sufficient to reverse that inhibition and trigger intense feeding behavior. We used 3D imaging and functional studies to illuminate the anatomical underpinning of both the inhibitory and excitatory events. Our work suggests that translational applications that aim to control appetite need to target the activation rather than the inhibition mechanisms. (See pp. E9489–E9498.)

ACSS2 promotes systemic fat storage and utilization through selective regulation of genes involved in lipid metabolism

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Animals lacking the ACSS2 enzyme are phenotypically normal when fed a standard chow diet. Surprisingly, when fed a high-fat diet, ACSS2-deficient animals become markedly less obese than their wild-type littermates. We further observe attenuation in the accumulation of fat in the livers of ACSS2-deficient mice. We show that the ACSS2 enzyme acts more like a transcription factor than a metabolic enzyme. It facilitates the dynamic reprogramming of gene expression in many different tissues to orchestrate the proper physiological adaptation of animals to the fed or fasted state. A potent and selective chemical inhibitor of the ACSS2 enzyme could represent a unique therapy for the treatment of both obesity and fatty liver disease. (See pp. E9499–E9506.)