PNAS Plus Significance Statements

Hydrodynamics and collective behavior of the tethered bacterium *Thiovulum majus*

Alexander Petroff and Albert Libchaber

In this paper we examine the dynamics underlying a form of collective behavior exhibited by bacteria. In a nutrient gradient, *Thiovulum majus* cells aggregate into a community in which cells attach to one another using mucus tethers. As tethered cells beat their flagella, they pull nutrient-laden water through the community. The flow of water created by many cells gives rise to variations in the nutrient concentration. As cells reorganize in response to these nutrient gradients, they change the flow of water. We show (pp. E537–E545) that the coupling between the motion of water, nutrients, and cells generates large-scale flows that dramatically increase the rate at which nutrients are pulled to the cells.

Role of clusters in nonclassical nucleation and growth of protein crystals

Mike Sleutel and Alexander E. S. Van Driessche

Intermediate metastable states are believed to be vital in the process of nucleation of crystalline material from solution. Our experimental evidence (pp. E546–E553) shows such intermediates can be liquid-like clusters that are stable with respect to the parent liquid and metastable compared with the emerging crystalline phase. Under given conditions, these clusters can contribute actively to the nucleation process, and hence, at least in the case for the proteins tested, partake in a two-step nucleation process. Moreover, upon merging with the crystal lattice, these clusters lead to a nonclassical mechanism of crystal growth that triggers a self-purifying cascade of impurity poisoned crystal surfaces.

Artificial riboswitches for gene expression and replication control of DNA and RNA viruses

Patrick Ketzer, Johanna K. Kaufmann, Sarah Engelhardt, Sascha Bossow, Christof von Kalle, Jörg S. Hartig, Guy Ungerechts, and Dirk M. Nettelbeck

Riboswitches are short RNA sequences for ligand-dependent modulation of gene expression *in cis*. This study (pp. E554–E562) demonstrates that an artificial riboswitch, a ligand-dependent self-cleaving ribozyme (aptazyme), can knockdown expression of an adeno- (DNA) virus early and a measles (RNA) virus structural gene, impacting biological outcomes, i.e. inhibiting viral genome replication and infectivity, respectively. It is the first report of riboswitches for replication control of human-pathogenic viruses and of their function in fully cytoplasmic (virus) systems. For future applications, aptazymes can be customized in other viruses facilitating analyses of viral gene functions or as a safety switch in oncolytic viruses. Because of their small size and RNA-intrinsic activity, we propose aptazymes as an alternative for inducible promoters in eukaryotic gene expression control.

Toward rationally redesigning bacterial two-component signaling systems using coevolutionary information

Ryan R. Cheng, Faruck Morcos, Herbert Levine, and José N. Onuchic

Our study (pp. E563–E571) uses amino acid coevolutionary information to better understand how bacterial two-component signaling (TCS) proteins preferentially interact with their correct partners while avoiding interactions with nonpartners. We extract coevolutionary couplings from sequences of TCS partners and study how coevolution is necessary to maintain their ability to transfer signals with high specificity. We use these coevolving couplings to devise a metric, which can predict the effects of mutations in the quality of signal transmission observed in vitro and provide support to the hypothesis that hybrid TCS proteins have reduced specificity. Our metric can potentially be used to redesign a TCS protein to preferentially interact with a nonpartner. Furthermore, our study can potentially be extended to networks of interacting proteins.

Induced multipotency in adult keratinocytes through down-regulation of *ΔNp63* or *DGCR8*

Deepavali Chakravarti, Xiaohua Su, Min Soon Cho, Ngoc Hoang Bao Bui, Cristian Coarfa, Avinashnarayan Venkatanarayan, Ashley L. Benham, Ramón E. Flores González, Jennifer Alana, Weimin Xiao, Marco L. Leung, Harina Vin, Io Long Chan, Arianexys Aquino, Nicole Müller, Hongran Wang, Austin J. Cooney, Jan Parker-Thornburg, Kenneth Y. Tsai, Preethi H. Gunaratne, and Elsa R. Flores

The p53 family member deltaNp63 (ΔNp63) is required for transcriptional activation of the microprocessor complex subunit DGCR8 in epidermal cells, leading to terminal differentiation of tissues such as the epidermis. We show here (pp. E572–E581) that loss of ΔNp63 leads to the generation of cells with self-renewing but limited differentiation capacity. When DGCR8 is reexpressed in cells deficient for ΔNp63, these cells can terminally differentiate into all three germ layers. We dubbed these cells induced multipotent stem cells because of their remarkable plasticity and ability to differentiate into multiple cell lineages. Based on our results using human keratinocytes, we predict that epidermal cells can be extracted from patient skin biopsies and reprogrammed into multipotent stem cells by knockdown of ΔNp63 or DGCR8.

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Sel1L is indispensable for mammalian endoplasmic reticulum-associated degradation, endoplasmic reticulum homeostasis, and survival

Shengyi Sun, Guojun Shi, Xuemei Han, Adam B. Francisco, Yewei Ji, Nuno Mendonça, Xiaojing Liu, Jason W. Locasale, Kenneth W. Simpson, Gerald E. Duhamel, Sander Kersten, John R. Yates III, Qiaoming Long, and Ling Qi

This study (pp. E582–E591) provides insights into the physiological role of Sel1L, an adaptor protein for the ubiquitin ligase Hrd1 in endoplasmic reticulum-associated degradation (ERAD). Using both animal and cell models, this study provides unequivocal evidence for an indispensable role of Sel1L in Hrd1 stabilization, mammalian ERAD, endoplasmic reticulum homeostasis, protein translation, and cellular and organismal survival. Moreover, generation of inducible knockout mouse and cell models deficient in both Sel1L and Hrd1 provides an unprecedented opportunity to elucidate the functional importance of this key branch of ERAD in vivo and to identify its physiological substrates.

Prostatic inflammation enhances basal-to-luminal differentiation and accelerates initiation of prostate cancer with a basal cell origin

Oh-Joon Kwon, Li Zhang, Michael M. Ittmann, and Li Xin

Inflammation promotes the initiation of various malignancies by inducing genetic and epigenetic changes. Here we show (pp. E592–E600) that bacterial infection-induced prostatitis results in microenvironmental changes that enhance the differentiation of prostate basal cells into luminal cells, a cellular process that rarely occurs under normal physiological conditions. Previously, we showed in a mouse model that disease initiation for prostate cancer with a basal cell origin requires and is limited by basal-to-luminal differentiation and that prostatic inflammation induced by bacterial infection accelerates disease initiation by enhancing basal-to-luminal differentiation. Collectively, our results show that inflammation-induced microenvironmental changes alter the prostate epithelial lineage differentiation program, and we propose this alteration as a distinct and complementary process through which inflammation promotes tumor initiation.

Elevated expression of TANK-binding kinase 1 enhances tamoxifen resistance in breast cancer

Congwen Wei, Yuan Cao, Xiaoli Yang, Zirui Zheng, Kai Guan, Qiang Wang, Yanhong Tai, Yanhong Zhang, Shengli Ma, Ye Cao, Xiaoxing Ge, Changzhi Xu, Jia Li, Hui Yan, Youguo Ling, Ting Song, Lin Zhu, Buchang Zhang, Quanbin Xu, Chengjin Hu, Xiu-wu Bian, Xiang He, and Hui Zhong

We investigated the possible role of TANK-binding kinase 1 (TBK1) protein in tamoxifen resistance and found that phosphorylation by TBK1 at the Ser-305 site stabilized estrogen receptor α (ERα) and modulated its transcriptional activity (pp. E601–E610). Ectopic expression of TBK1 rendered breast cancer cells resistant to tamoxifen. TBK1 inhibition sensitized breast cancer cells to tamoxifen-induced cell death. The expression of TBK1 was increased in subjects with breast cancer and was positively correlated with ERα, ERα Ser-305, and cyclin D1 expression. Subjects with tumors that highly expressed TBK1 had poor responsiveness to tamoxifen treatment. Therefore, TBK1 is potentially a unique predictive marker of tamoxifen resistance and a potential therapeutic target for breast cancer.

HSV-1 degrades, stabilizes, requires, or is stung by STING depending on ICP0, the US3 protein kinase, and cell derivation

Maria Kalamvoki and Bernard Roizman

STING (stimulator of IFN genes) and IFI16 are sensors of DNA in cytoplasm and the nucleus, respectively. Both signal activation of innate immune responses. Both proteins are stable in wild-type virus-infected cells. The key finding (pp. E611–E617) with broad implications is that in HSV-1–infected cells STING and IFI16 are actively stabilized inasmuch as they are degraded in cells lacking functional ICP0 or protein kinase US3. Moreover, STING was required for optimal replication in HEp-2 or HeLa cells derived from cancers but was inimical in HEL or HEK293 cells derived initially from normal tissues and in the case of HEK293 cells transformed with viral oncogenes. The requirements for stabilization of STING and IFI16 were not covariant.

Decision-related pupil dilation reflects upcoming choice and individual bias

Jan Willem de Gee, Tomas Knapen, and Tobias H. Donner

A number of studies reported that the pupil dilates (under constant illumination) during decision-making. Pupil dilation is also associated with the brain-wide release of modulatory neurotransmitters. It has remained unknown which specific elements of decision processes drive pupil dilation. Using a visual detection task, we here show (pp. E618–E625) that pupil dilation is primarily driven during, and not at the end of, a protracted decision. Further, pupil dilation differentiates between "yes" and "no" choices for conservative subjects deciding yes against their bias. Thus, pupil dilation reveals the content of the evolving decision and the decision maker's attitude. These findings have important implications for interpreting decision-related brain activity. They also point to a possible role of neuromodulation in interacting with decision biases.

Parameterizing cell-to-cell regulatory heterogeneities via stochastic transcriptional profiles

Sameer S. Bajikar, Christiane Fuchs, Andreas Roller, Fabian J. Theis, and Kevin A. Janes

Cell-to-cell variations in gene regulation occur in a number of biological contexts, such as development and cancer. Discovering regulatory heterogeneities in an unbiased manner is difficult owing to the population averaging that is required for most global molecular methods. Here (pp. E626–E635), we show that we can infer single-cell regulatory states by mathematically deconvolving global measurements taken as averages from small groups of cells. This averaging-and-deconvolution approach allows us to quantify single-cell regulatory heterogeneities while avoiding the measurement noise of global single-cell techniques. Our method is particularly relevant to solid tissues, where single-cell dissociation and molecular profiling is especially problematic.