PNAS Plus Significance Statements

Uncovering hidden variation in polyploid wheat

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Pasta and bread wheat are polyploid species that carry multiple copies of each gene. Therefore, loss-of-function mutations in one gene copy are frequently masked by functional copies on other genomes. We sequenced the protein coding regions of 2,735 mutant lines and developed a public database including more than 10 million mutations. Researchers and breeders can search this database online, identify mutations in the different copies of their target gene, and request seeds to study gene function or improve wheat varieties. Mutations are being used to improve the nutritional value of wheat, increase the size of the wheat grains, and generate additional variability in flowering genes to improve wheat adaptation to new and changing environments. (See pp. E913–E921.)

Differential control of retrovirus silencing in embryonic cells by proteasomal regulation of the ZFP809 retroviral repressor

Cheng Wang and Stephen P. Goff

A key transcriptional silencer responsible for suppression of retroviral gene expression in embryonic stem cells is found to be regulated itself at the level of protein turnover, mediated by ubiquitinylation and proteasomal degradation. The implication is that the stem cell state is characterized not only by an unusual transcriptional profile but also by unusual posttranscriptional regulation. (See pp. E922–E930.)

Mutation of a kinase allosteric node uncouples dynamics linked to phosphotransfer

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Our Girvan–Newman-based community network analysis provides a strategy to explore the effects of protein kinase mutations on the dynamic properties of the kinase core and the allosteric network underlying the catalytic cycle. With this approach, which allows us to identify subtle shifts in dynamics and in particular side-chain rotamer preferences, we can explain the underlying mechanism for loss of function in many different scenarios. Our method is especially relevant for disease-causing mutations that lie far from the active site but show no obvious structural perturbations based on X-ray crystallography. (See pp. E931–E940.)

Heat-induced masculinization in domesticated zebrafish is family-specific and yields a set of different gonadal transcriptomes

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Fish exhibit remarkable sexual plasticity. However, the underlying mechanism of heat-induced sex reversal is still unclear. Here we first established the conditions for heat-induced reprogramming of sexual phenotypes in zebrafish through sex ratio analysis and gonad transcriptomics. Sex ratio response to heat was family-specific and resulted in masculinization. We observed two heat-induced gonadal transcriptomic profiles per sex in adults, among them neomales and, strikingly, females with an ovary but a "male-like" transcriptome. The latter indicates major transcriptomic reprogramming with preserved organ structure, an interesting observation in vertebrates. In all heat-treated juveniles, we also observed a male-like transcriptome. Overall, this study reveals novel lasting thermal effects on fish gonads, with practical implications for studying the effects of global warming in natural populations. (See pp. E941–E950.)

Similarities and differences in the transcriptional control of expression of the mouse TSLP gene in skin epidermis and intestinal epithelium

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Thymic stromal lymphopoietin (TSLP) is a critical immunoregulatory cytokine that plays important physiological functions in epithelial cells in skin and intestinal barriers. However, the molecular mechanisms controlling TSLP expression in vivo are still poorly understood. Using tissue-selective mutagenesis in mice, we have identified the involvement of multiple transcriptional factors, including several nuclear receptors and their cognate agonistic ligands, in the transcriptional regulation of TSLP in epidermal keratinocytes and intestinal epithelial cells. Importantly, this investigation also demonstrates that the retinoid X receptor (RXR) and retinoic acid receptor (RAR) isotypes are not functionally redundant in

vivo. Taking our data together, the present study unveils the topological map and the combinatorial mechanisms involved in tissue-specific transcriptional regulation of TSLP expression in epidermal keratinocytes and intestinal epithelial cells. (See pp. E951–E960.)

Active MLKL triggers the NLRP3 inflammasome in a cell-intrinsic manner

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Necroptotic cell death is mediated by activation of the mixed-lineage kinase domain-like protein (MLKL). The inflammation associated with this form of cell death is thought to be due to the release of proinflammatory cellular contents after plasma membrane rupture. In contrast to this prevailing view, we show that MLKL activates the innate immune receptor nucleotide-binding oligomerization domain (NOD)-like receptor protein 3 (NLRP3) in a cell-intrinsic manner. Importantly, we show that MLKL-mediated NLRP3 and caspase-1 activation and the secretion of the proinflammatory cytokine IL-1β is a major determinant of necroptotic-derived inflammatory signals. These findings suggest that NLRP3 and IL-1β may be relevant therapeutic targets in MLKL-driven diseases. (See pp. E961–E969.)

Immunoinhibitory checkpoint deficiency in medium and large vessel vasculitis

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Antigen recognition by the immune system triggers rapid, specific, and protective responses, which are counterbalanced by inhibitory checkpoints to minimize potentially harmful immunity. The programmed death-1/ programmed death ligand-1 (PD-1/PD-L1) checkpoint is overreactive in cancer patients, curbing antitumor immunity. Whether a failing PD-1/PD-L1 checkpoint contributes to spontaneous autoimmune disease in humans is unknown. Here, we found that in patients with the autoimmune vasculitis giant cell arteritis, antigen-presenting cells provide insufficient negative signaling; unleashing highly activated T cells to infiltrate and damage the walls of large arteries. Thus, immunoinhibitory signals protect large arteries against inflammatory attack and checkpoint activation may be a suitable strategy to treat autoimmune vasculitis. (See pp. E970–E979.)

CD4 T-cell cytokines synergize to induce proliferation of malignant and nonmalignant innate intraepithelial lymphocytes

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Refractory celiac disease type II (RCDII) is a severe variant of celiac disease, an autoimmune disorder of the small intestine caused by inflammatory T-cell responses to gluten, a common food protein. Typical of RCDII is the presence of aberrant lymphocytes in the duodenal epithelium, which often give rise to a lethal lymphoma. A single growth factor promoting the expansion of aberrant cells has been identified: epithelial cell-derived IL-15. The experiments described in this paper identify three additional growth factors—TNF, IL-2, and IL-21—produced by gluten-specific T cells. Thus, these findings suggest a potential mechanism for the contribution of gluten-specific T cells to RCDII. (See pp. E980–E989.)

Induction of dormancy in hypoxic human papillomavirus-positive cancer cells

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Human papillomaviruses (HPVs) are major human carcinogens. It is widely assumed that HPV-positive tumor cells must sustain viral *E6/E7* oncogene expression to continuously block the tumor-suppressive senescence response of the host cell. Consequently, E6/E7 are considered attractive therapeutic targets for immunotherapy or for functional inhibition. Here we show that hypoxic conditions, as often found in HPV-positive cancers, allow the cells to induce a dormant state in which E6/E7 is down-regulated but induction of senescence is avoided. Instead, a reversible growth arrest is induced that can be overcome by reoxygenation. As a consequence, hypoxic HPV-positive cancer cells are protected against chemotherapy as well as against virus-specific therapeutic approaches, and may serve as reservoirs for cancer recurrence on reoxygenation. (See pp. E990–E998.)

Interference of the complex between NCS-1 and Ric8a with phenothiazines regulates synaptic function and is an approach for fragile X syndrome

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Neurons coregulate their number of synapses and the probability of neurotransmitter release per synapse in an antagonistic manner. The binding of neuronal calcium sensor 1 (NCS-1) to the guanine exchange factor protein Ric8a coregulates these neuronal features. This study identified a small molecule, the phenothiazine FD44, that binds the interaction surface between NCS-1 and Ric8a, preventing the formation of the complex. Tested on a *Drosophila* model of the fragile X syndrome, where the number of synapses is in excess, FD44 proves effective to reduce synapse number to normal levels and restore normal learning performance. Our structure–function study shows the specificity of this compound and the drugability of the NCS-1/Ric8a interface for the treatment of fragile X and possibly, other synaptopathies. (See pp. E999–E1008.)

A natural product inhibits the initiation of α-synuclein aggregation and suppresses its toxicity

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Parkinson's disease is characterized by the presence in brain tissues of aberrant aggregates primarily formed by the protein α-synuclein. It has been difficult, however, to identify compounds capable of preventing the formation of such deposits because of the complexity of the aggregation process of α-synuclein. By exploiting recently developed highly quantitative in vitro assays, we identify a compound, squalamine, that blocks α-synuclein aggregation, and characterize its mode

of action. Our results show that squalamine, by competing with α-synuclein for binding lipid membranes, specifically inhibits the initiation of the aggregation process of α-synuclein and abolishes the toxicity of α-synuclein oligomers in neuronal cells and in an animal model of Parkinson's disease. (See pp. E1009–E1017.)

Forkhead box a2 (FOXA2) is essential for uterine function and fertility

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These studies define biological roles for Forkhead box a2 (FOXA2) and glands of the uterus in female reproduction and fertility. FOXA2 is a critical regulator of uterine gland development in the neonate as well as of differentiated gland function in the adult uterus. Our findings provide clear in vivo evidence that FOXA2 regulates uterine expression of Leukemia inhibitory factor (Lif) for blastocyst implantation and that uterine glands play active roles in stromal cell decidualization and placental development. These findings support the idea that pregnancy loss and complications in women may have their origin in uterine gland dysfunction. (See pp. E1018–E1026.)

Endosperm-based hybridization barriers explain the pattern of gene flow between *Arabidopsis lyrata* and *Arabidopsis arenosa* in Central Europe

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Hybrid seed lethality has been recognized and addressed by a long-standing tradition of plant-breeding research. Nevertheless, its role in evolution and speciation has been underestimated. In this study, hybrid seed lethality between *Arabidopsis lyrata* and *Arabidopsis arenosa*, two model species of growing interest in the scientific community, was investigated. This study shows that endosperm defects are sufficient to explain the direction of gene flow between the two wild species, suggesting an important role of this hybridization barrier in plant speciation. In addition, we show that natural polyploidization is involved in breaking down hybridization barriers, not only establishing them, as implied by the traditional "triploid block" concept. Our data suggest that polyploidy-mediated hybrid seed rescue, long known in artificial crosses, could play an important role in plant evolution. (See pp. E1027–E1035.)