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INVESTIGATIONS

- 1619–1628 **Mono and Dual Cofactor Dependence of Human Cystathionine β -Synthase Enzyme Variants *In Vivo* and *In Vitro***
Dago Dimster-Denk, Katherine W. Tripp, Nicholas J. Marini, Susan Marqusee, and Jasper Rine
Variation in gene sequences among humans includes SNPs that cause nonsynonymous amino acid substitutions. These substitutions can alter the function of a protein. Some enzymes can compensate for the impact of the substitution with increased levels of cofactors. Such cofactor remedial alleles are perhaps the most accessible dimension of personalized genetics. In this study, the authors created and evaluated a reference set of alleles in an enzyme with two different cofactors to explore the principles behind cofactor remediation.
- 1629–1637 **A Large-Scale Behavioral Screen to Identify Neurons Controlling Motor Programs in the *Drosophila* Brain**
Thomas F. Flood, Michael Gorczyca, Benjamin H. White, Kei Ito, and Motojiro Yoshihara
These authors performed a thermo-genetic screen to find and characterize neural circuits by expressing neuronal activators to stimulate subsets of neurons to induce behavior. Using the large collection of Gal4 lines from the NP project, they crossed 835 Gal4 strains to flies carrying a UAS-transgene encoding TRPM8, a cold-sensitive ion channel. Low temperatures opened the TRPM8 channel in Gal4 expressing cells, causing excitation and in many cases inducing overt behavioral changes in adult flies. The authors also performed stimulation using the heat-activated channel TrpA1. This resulted in clearer and more robust behaviors including flight, feeding, and egg laying.
- 1639–1647 **The Components of *Drosophila* Histone Chaperone dCAF-1 Are Required for the Cell Death Phenotype Associated with *rbf1* Mutation**
Heather Collins and Nam-Sung Moon
Through investigating the genetic interaction between Psc and *rbf1*, the authors discovered that CAF1p55 function is commonly affected by Psc and *rbf1*. CAF1p55 is a shared component of numerous chromatin-associated protein complexes such as NuRD, dREAM/MMV, NURF, and dCAF-1. Here the authors provide evidence suggesting that Psc activation antagonizes CAF1p55 function, and that dCAF-1 function is deregulated in *rbf1* mutant cells. These findings raise the possibility that Psc and Rbf1 may have epigenetic consequences that were previously unappreciated.
- 1649–1659 **Yeast hEST1A/B (SMG5/6)-Like Proteins Contribute to Environment-Sensing Adaptive Gene Expression Responses**
Xianning Lai, Traude Beilharz, Wei-Chun Au, Andrew Hammet, Thomas Preiss, Munira A. Basrai, and Jörg Heierhorst
hEST1A/B (SMG5/6)-like proteins play important roles in telomere and NMD pathways in metazoans, but their evolutionary origin is unclear. Here the authors identify two new members of this gene family in budding yeast that do not share NMD or telomere functions but instead contribute to environment sensing transcriptional responses. The results indicate a broader range of cellular function of this gene family than previously anticipated.

- 1661–1674 **The C-terminal Residues of *Saccharomyces cerevisiae* Mec1 Are Required for Its Localization, Stability, and Function**
Lance F. DaSilva, Samantha Pillon, Julie Genereaux, Megan J. Davey, Gregory B. Gloor, Jim Karagiannis, and Christopher J. Brandl
- PIKK proteins are critical in the response of eukaryotic cells to environmental change and stress. Mec1/ATR is essential for the DNA damage response and DNA repair. Understanding its structure/function is relevant to signaling pathways and drug design. In this article, the authors detail an essential function for the C-terminus of Mec1 (the FATC domain) in the protein's folding/stability and localization. Manipulating this domain and those of other PIKK proteins is thus a mechanism to regulate their function. Using an unbiased genetic screen, the authors also found evidence that the proteasome regulates Mec1 levels and that Rpn3 has a key role in this function.
- 1675–1686 **Expression Plasmids for Use in *Candida glabrata***
Rebecca E. Zordan, Yuxia Ren, Shih-Jung Pan, Giuseppe Rotondo, Alejandro De Las Peñas, Joseph Iluore, and Brendan P. Cormack
- In this study, the authors construct and validate a series of episomal expression plasmids for use in the yeast *Candida glabrata*. The vectors contain a choice of promoters and are available on backbones with either *URA3*- or nourseothricin-resistance markers. The available promoters allow for a range of constitutive (*EGD2pr*, *HHT2pr*, *PDC1pr*), phagocytosis-induced (*ACO2pr*, *LYS21pr*), or nutritionally-regulated (*MET3pr*) expression. The authors measured expression in each plasmid configuration using GFP as a reporter and monitored expression via flow cytometry, microscopy, and quantitative reverse transcription PCR.
- 1687–1695 **A Deep Intronic Mutation in the Ankyrin-1 Gene Causes Diminished Protein Expression Resulting in Hemolytic Anemia in Mice**
Hua Huang, PengXiang Zhao, Kei Arimatsu, Koichi Tabeta, Kazuhisa Yamazaki, Lara Krieg, Emily Fu, Tian Zhang, and Xin Du
- The laboratory mouse is a superior model system to study human genetic diseases. It offers unique insight into the molecular details of pathogenesis. These authors identified a mouse phenotype of hemolytic anemia in a forward genetic screen. Positional cloning and characterization of the phenotype revealed an intriguing RNA splicing defect of the *Ank1* gene in the mutant. The defect resulted in diminished ANK1 protein expression rather than producing aberrant protein. The authors have therefore established a unique animal model for the study of the quantitative impact of ankyrin-1 protein on the development of hemolytic anemia.
- 1697–1705 **Targeted Mutagenesis of *Arabidopsis thaliana* Using Engineered TAL Effector Nucleases**
Michelle Christian, Yiping Qi, Yong Zhang, and Daniel F. Voytas
- TALENs have recently emerged as powerful reagents for creating targeted modifications in a variety of eukaryotic genomes. These authors used TALENs to create targeted loss-of-function mutations in the model plant *Arabidopsis thaliana*. This mutagenesis approach should facilitate genetic analysis of the many *Arabidopsis* genes of unknown function.
- 1707–1715 **Targeted Deletion and Inversion of Tandemly Arrayed Genes in *Arabidopsis thaliana* Using Zinc Finger Nucleases**
Yiping Qi, Xiaohong Li, Yong Zhang, Colby G. Starker, Nicholas J. Baltes, Feng Zhang, Jeffry D. Sander, Deepak Reyon, J. Keith Joung, and Daniel F. Voytas
- Duplicate genes frustrate genetic analyses. In this article, the authors describe an approach to mutate tandemly duplicated genes in *Arabidopsis*, an arrangement that characterizes 17% of the genes in this important model plant species.
- 1717–1725 **Comparing Zinc Finger Nucleases and Transcription Activator-Like Effector Nucleases for Gene Targeting in *Drosophila***
Kelly J. Beumer, Jonathan K. Trautman, Michelle Christian, Timothy J. Dahlem, Cathleen M. Lake, R. Scott Hawley, David J. Grunwald, Daniel F. Voytas, and Dana Carroll
- TALENs and ZFNs are targetable nucleases that have been used for genome engineering. In this study, the authors directly compared these two platforms in *Drosophila melanogaster*. They found that TALENs are easier to design for new targets and more reliably active, although not all designs are successful. They also report on parameters that affect TALEN activity and the types of mutations induced.

- 1727–1740 **Cytoplasmic Male Sterility Contributes to Hybrid Incompatibility Between Subspecies of *Arabidopsis lyrata***
Esa A. Aalto, Hans-Peter Koelewijn, and Outi Savolainen
 Hybrids between diverged populations may suffer reduced fitness due to genomic incompatibilities. The authors first confirm that differences in cytoplasmic male sterility (CMS) and fertility restorer (*rf*) genes cause asymmetrical hybrid incompatibility between two Lyrate rockcross (*Arabidopsis lyrata*) populations belonging to different subspecies. They go on to show the importance of transmission ratio distortion when examining inheritance based on genotype ratios. Finally, the authors map the *rf* and show that there is no trade-off between the CMS and seed production. The observed incompatibility has limited contribution to incipient speciation, but may be an interesting part of metapopulation dynamics in many plant species.
- 1741–1751 **Global Linkage Map Connects Meiotic Centromere Function to Chromosome Size in Budding Yeast**
Anastasia Baryshnikova, Benjamin VanderSluis, Michael Costanzo, Chad L. Myers, Rita S. Cha, Brenda Andrews, and Charles Boone
 These authors constructed a genome-wide genetic linkage map for the budding yeast *Saccharomyces cerevisiae* by examining the frequency of forming double mutants for ~1.2 million pairs of loci occurring on the same chromosome. The map highlights recombination hotspots and shows that the extent of suppression of recombination across pericentric regions correlates positively with chromosome size. Centromeres of larger chromosomes also appear to load more extensive clusters of meiotic cohesin and acquire fewer DNA double strand breaks. These correlations suggest that chromosome size may impact meiosis-specific centromere structure and function and ultimately influence the incidence of aneuploidy.
- 1753–1758 **Characterization of Two ENU-Induced Mutations Affecting Mouse Skeletal Morphology**
Shauna M. Dauphinee, Megan M. Eva, Kyoko E. Yuki, Melissa Herman, Silvia M. Vidal, and Danielle Malo
 This article describes the congenital skeletal deformities observed in two mutant mouse strains generated from ENU mutagenesis. The genes underlying these mutants, *Npr3* and *Flnb*, have previously been associated with skeletal abnormalities, making these ENU mutants useful models for the study of vertebral malformations.
- 1759–1767 **A Whole-Genome DNA Marker Map for Cotton Based on the D-Genome Sequence of *Gossypium raimondii* L.**
Zining Wang, Dong Zhang, Xiyin Wang, Xu Tan, Hui Guo, and Andrew H. Paterson
 These authors have created a whole genome marker map for cotton that integrates information from DNA markers, QTLs, and the reference genome sequence. Its density of one locus per 15.6 kb is five times higher than the most dense single published map of cotton. The map is a versatile tool for molecular breeding, fine mapping and cloning of genes and QTLs, developing new genetic markers and maps, genome wide association mapping, and genome evolution studies. It provides a general platform for marker and trait study and will facilitate molecular breeding and other studies in the cotton community.
- 1769–1777 **Disruption of the Rice Plastid Ribosomal Protein S20 Leads to Chloroplast Developmental Defects and Seedling Lethality**
Xiaodi Gong, Quan Jiang, Jianlong Xu, Jianhui Zhang, Sheng Teng, Dongzhi Lin, and Yanjun Dong
 Plastid ribosomal proteins (PRPs) are essential for ribosome biogenesis, plastid protein biosynthesis, chloroplast differentiation and early chloroplast development. This study identifies the first rice PRP mutant, *asl1* (albino seedling lethality1), which exhibits an albino lethal phenotype at the seedling stage. This albino phenotype was associated with altered chlorophyll (Chl) content and chloroplast development. Map-based cloning revealed that *ASL1* encodes plastid ribosomal protein S20 (PRPS20), which localizes to the chloroplast. *ASL1* showed tissue-specific expression, as it was highly expressed in plumule and young seedlings but expressed at much lower levels in other tissues. In addition, *ASL1* expression was regulated by light. The transcript levels of nuclear genes for Chl biosynthesis and chloroplast development were strongly affected in *asl1* mutants; transcripts of some plastid genes for photosynthesis were undetectable. The authors' findings indicate that nuclear-encoded PRPS20 plays an important role in chloroplast development in rice.

- 1779–1784 **Identification of Genes Interacting with *rnt-1* Through Large-Scale RNAi Screening in *Caenorhabditis elegans***
Kiho Lee, Jiwon Shim, Jihyun Lee, and Junho Lee
 To determine how RUNX proteins work together with other cofactors, the authors identified genes that genetically interact with RNT-1 in *C. elegans* by a large scale synthetic RNAi phenotype screen. They found that CDK8, a component of a transcriptional mediator, caused a severe phenotype only in *rnt-1* mutant background. The study also identified a putative target gene of RNT-1/CDK-8 complex.
- 1785–1794 **The Drosophila *Enhancer of split* Gene Complex: Architecture and Coordinate Regulation by Notch, Cohesin, and Polycomb Group Proteins**
Cheri A. Schaaf, Ziva Misulovin, Maria Gause, Amanda Koenig, and Dale Dorsett
 This study shows that cohesin and the PRC1 Polycomb complex control the association of the Notch activator protein with the *Enhancer of split* gene complex. This gene complex has a higher order chromosomal architecture that likely dictates the binding and action of cohesin and PRC1. These findings are relevant to understanding how chromosome structure controls gene expression and the molecular basis of human cohesinopathies.
- 1795–1807 **Imputation-Based Genomic Coverage Assessments of Current Human Genotyping Arrays**
Sarah C. Nelson, Kimberly F. Doheny, Elizabeth W. Pugh, Jane M. Romm, Hua Ling, Cecelia A. Laurie, Sharon R. Browning, Bruce S. Weir, and Cathy C. Laurie
 Choosing from among the many available SNP microarrays can present a practical challenge to researchers planning human genetic studies. To aid these decisions, the authors have estimated the genomic coverage of eight currently available arrays using genome-wide imputation and phase 1 of the 1000 Genomes Project. Coverage is presented separately by continental ancestry group and across different minor allele frequency ranges. The authors also show estimated power to detect trait-variant associations with each array, given the level of genomic coverage. The results of this study can help researchers choose from among available arrays based on their genotyping budget, number of available samples, and overall research goals.
- 1809–1818 **Insights into the Evolution of Cotton Diploids and Polyploids from Whole-Genome Re-sequencing**
Justin T. Page, Mark D. Huynh, Zach S. Liechty, Kara Grupp, David Stelly, Amanda M. Hulse, Hamid Ashrafi, Allen Van Deynze, Jonathan F. Wendel, and Joshua A. Udall
 In this study, the authors aligned resequencing reads to the existing D-genome sequence and discovered novel changes between the A- and D-genomes in both diploid and polyploid plants. They identified single base differences throughout the genome between the diploid genomes and discovered that 978 genes of the D-genome reference sequence are consistently deleted in the A-genome. They also discovered that ~900Kbp of sequence in the polyploid genome has been converted from one genome to another in separate conversion events scattered across the genome. These discoveries will lead to a better understanding of the dynamic nature of polyploid genomes and provide many avenues for further genomic research in cotton.
- 1819–1825 **Identification of a Novel Polymorphism in X-Linked Sterol-4-Alpha-Carboxylate 3-Dehydrogenase (*Nsdhl*) Associated with Reduced High-Density Lipoprotein Cholesterol Levels in I/LnJ Mice**
David J. Bautz, Karl W. Broman, and David W. Threadgill
 The authors have identified a novel HDL QTL on the X chromosome that harbors a polymorphism in *Nsdhl* that is associated with a decreased level of HDL cholesterol. This suggests that additional loci controlling HDL levels have yet to be identified that could provide novel targets for therapeutic intervention.
- 1827–1832 **Efficient Single-Cell Transgene Induction in *Caenorhabditis elegans* Using a Pulsed Infrared Laser**
Matthew A. Churgin, Liping He, John I. Murray, and Christopher Fang-Yen
 The targeting of transgene expression to specific cells is often limited by the availability of appropriate promoters. In this study, the authors present a method for evoking gene expression in arbitrary cells in *C. elegans*. They use a focused pulsed infrared laser to locally induce a heat shock response in targeted cells. Their method expands upon previously-reported techniques involving continuous-wave lasers. Pulsed laser illumination enables a much higher degree of spatial selectivity. The authors use their method to induce transient and long-term transgene expression in a variety of cell types.

- 1833–1842 **Genetic Architecture of Parallel Pelvic Reduction in Ninespine Sticklebacks**
Takahito Shikano, Veronika N. Laine, Gábor Herczeg, Johanna Vilkki, and Juha Merilä
 The relative importance of standing genetic variation and new mutations in evolutionary processes is a long-standing question. In general, the likelihood of genetic parallelism underlying similar phenotypic changes is thought to decrease with increasing evolutionary distance between taxa. In contrast, based on the inter- and intra-specific comparative mapping analyses of pelvic reduction in sticklebacks, the authors show alternative genetic mechanisms resulting in similar phenotypes can evolve over a short evolutionary time scale, thereby providing important insight into the genetic basis and evolutionary processes behind parallel phenotypic changes.
- 1843–1850 **Functional Conservation of *Gsdma* Cluster Genes Specifically Duplicated in the Mouse Genome**
Shigekazu Tanaka, Youichi Mizushima, Yoriko Kato, Masaru Tamura, and Toshihiko Shiroishi
 Mouse *Gsdma3* is the causative gene for alopecia. In this study, the authors found that tandem gene duplication occurred only in the mouse lineage, and the mouse has two other *Gsdma3*-related genes, *Gsdma* and *Gsdma2*. To date, no skin mutation has been found for human *GSDMA* and rat *Gsdma* as well as mouse *Gsdma*. To elucidate functional divergence among the *Gsdma*-related genes in mice, the authors generated *Gsdma* knockout mice and *Gsdma* transgenic mice. The results of this study indicate conservation of the *in vivo* function of mouse *Gsdma* and *Gsdma3* for regulating epithelial maintenance and/or homeostasis, and suggest that the human *GSDMA* and rat *Gsdma* have similar functions.
- 1851–1859 **When Females Produce Sperm: Genetics of *C. elegans* Hermaphrodite Reproductive Choice**
Adam K. Bahrami and Yun Zhang
C. elegans hermaphrodites are somatic females that evolved to produce sperm, which gives them the ability to self-reproduce or mate with males. This raises the possibility of strategic reproductive decision making. The authors found that hermaphrodites of the reference strain resist mating in favor of self-reproduction, and wild isolates exhibit substantial heritable variation in hermaphrodite mating. Their study decodes genetic and neuronal requirements of hermaphrodite reproductive choice and demonstrates that self-reproduction promotes resistance to mating. These findings contribute to revision of a prominent notion that *C. elegans* hermaphrodites are passive recipients of male mating and lay the groundwork for mechanistic dissection of this evolutionarily important trait.
- 1861–1867 **Practical Considerations Regarding the Use of Genotype and Pedigree Data to Model Relatedness in the Context of Genome-Wide Association Studies**
Riyan Cheng, Clarissa C. Parker, Mark Abney, and Abraham A. Palmer
 GWAS is a powerful tool for dissecting the genetic basis of quantitative traits; however, familial relatedness and population structure must be properly modeled to yield valid conclusions. This can be done using a pedigree, if available, but could potentially also be done using observed genotype data. In this article, the authors explore issues of type I error and power as it relates to using these two types of data.
- MUTANT SCREEN REPORTS**
- 1869–1873 ***Schizosaccharomyces japonicus* Yeast Poised to Become a Favorite Experimental Organism for Eukaryotic Research**
Amar J. S. Klar
 Despite generation times of up to two hours and genetic crosses that require seven days to complete, the budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe* are commonly used for research. In this article, the author predicts that the *Schizosaccharomyces japonicus* fission yeast will soon become a choice organism for research on the biology of eukaryotes. Though unrelated to *S. cerevisiae* and *S. pombe*, *S. japonicas* has a generation time of only 63 minutes, and meiotic analysis can be completed in just 60 hours.
- 1875–1880 ***Drosophila* Embryonic Cell-Cycle Mutants**
Yingdee Unhavaithaya, Eugenia A. Park, Irena Royzman, and Terry L. Orr-Weaver
 This mutant report details a valuable collection of mutations in genes encoding cell cycle regulators and essential cell division functions. Prior to this screen only a small number of *Drosophila* mutants with defective embryonic cell divisions had been isolated. These authors have identified mutants disrupting two cytokinesis proteins and two centromere/kinetochore proteins that will be useful in analysis of cell division. The alleles of *cyclin E* recovered also allowed the authors to define its instrumental role in neuroblast cell fate determination.