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INVESTIGATIONS

- 1–8 **5-Hydroxymethylcytosine Is Not Present in Appreciable Quantities in *Arabidopsis* DNA**
Robert M. Erdmann, Amanda L. Souza, Clary B. Clish, and Mary Gehring
 Modified DNA bases have essential biological functions. In animals, cytosine can be modified to 5-methylcytosine and further oxidized to 5-hydroxymethylcytosine. 5-methylcytosine is an abundant base within plant genomes. Erdmann *et al.* examined whether 5-hydroxymethylcytosine is found within the genome of *Arabidopsis thaliana*. The authors determined that 5-hydroxymethylcytosine is not found in plant genomic DNA in biologically relevant quantities. Although 5-hmC is thought to play a role in DNA demethylation within animals, it appears plants likely rely solely upon the established DNA glycosylase / base excision repair pathway to actively demethylate DNA.
- 9–19 ***In Silico* Scrutiny of Genes Revealing Phylogenetic Congruence with Clinical Prevalence or Tropism Properties of *Chlamydia trachomatis* Strains**
Rita Ferreira, Minia Antelo, Alexandra Nunes, Vítor Borges, Vera Damião, Maria José Borrego, and João Paulo Gomes
 Microbes use a diverse range of virulence factors to infect dissimilar tissues or hosts. The identification of such genes usually relies on the use of genetic manipulation. For the obligate intracellular human pathogen *Chlamydia trachomatis*, which is capable of infecting the ocular tissue, the genitalia, and also the phagocytes, knockout assays are still not straightforward, hampering the identification of such genes. Thus, the authors used bioinformatics to identify genes for which the mutational pattern is exclusive of the strains infecting the same tissue. This study may provide a genetic database for functional studies targeting disease-associated genes.
- 21–33 **Relationships of RNA Polymerase II Genetic Interactors to Transcription Start Site Usage Defects and Growth in *Saccharomyces cerevisiae***
Huiyan Jin and Craig D. Kaplan
 Alterations in the RNA Polymerase II (Pol II) active site trigger loop (TL) alter its catalytic properties and lead to transcription start site (TSS) distribution changes and growth defects *in vivo*. Jin and Kaplan investigate whether initiation defects in TL alleles as measured through TSS defects are the primary causes of their growth defects. The authors explore the relationships between Pol II alleles and factor mutants that show either wide-ranging genetic interactions with Pol II alleles or are themselves TSS defective. They find that in some cases growth defects can be separated from severity of initiation defects.
- 35–44 **Expression of *MTAP* Inhibits Tumor-Related Phenotypes in HT1080 Cells via a Mechanism Unrelated to Its Enzymatic Function**
Baiqing Tang, Yuwaraj Kadariya, Yibai Chen, Michael Slifker, and Warren D. Kruger
 Methylthioadenosine Phosphorylase (MTAP) is a basic metabolic enzyme that is also a tumor suppressor gene. Tang *et al.* show that in a MTAP-deleted fibrosarcoma cell line, expression of MTAP causes the cells to behave in a less tumorigenic manner, affecting the mRNA levels of hundreds of genes. However, these same effects are also observed when the MTAP-expressing cells are treated with a drug that inhibits MTAP's known enzymatic function, and when a catalytically inactive MTAP is introduced. The results show that MTAP's anti-tumorigenic effects are not mediated via its known enzymatic function.

- 45–48 **How Big Is Your Y? A Genome Sequence–Based Estimate of the Size of the Male-Specific Region in *Megaselia scalaris***
Kenneth B. Hoehn and Mohamed A. F. Noor
 A major question in evolutionary genetics is how sex chromosomes (like our X and Y) evolve. *Megaselia scalaris* is a pest species that purportedly has a primitive system in this regard, bearing a male-determining region on one chromosome rather than distinct sex chromosomes. Curiously, this male-determining region has been shown to “jump” between chromosomes in the laboratory. Hoehn and Noor use genome sequences to estimate the size of this region to determine if it corresponds with what is expected from known transposable elements. They find it to be unusually large and likely to have been stable in natural populations.
- 49–59 **Developmental Ethanol Exposure Leads to Dysregulation of Lipid Metabolism and Oxidative Stress in *Drosophila***
Theresa Logan-Garbisch, Anthony Bortolazzo, Peter Luu, Audrey Ford, David Do, Payam Khodabakhshi, and Rachael L. French
 Alcohol exposure during development causes an array of physical and neurobehavioral deficits, in humans collectively referred to as “Fetal Alcohol Effects” (FAE). Logan-Garbisch *et al.* have established a *Drosophila* model of FAE, and here show that developmental ethanol exposure causes a widespread disruption of lipid metabolism concomitant with increased oxidative stress and changes in antioxidant gene expression. The data suggest for the first time that increased lipid storage and the inability to properly metabolize long-chain fatty acids is one mechanism by which alcohol causes oxidative stress.
- 61–72 **Functional Variants in *DPYSL2* Sequence Increase Risk of Schizophrenia and Suggest a Link to mTOR Signaling**
Yaping Liu, Xuan Pham, Lilei Zhang, Pei-lung Chen, Grzegorz Burzynski, David M. McGaughey, Shan He, John A. McGrath, Paula Wolyniec, Margaret D. Fallin, Megan S. Pierce, Andrew S. McCallion, Ann E. Pulver, Dimitrios Avramopoulos, and David Valle
 Liu *et al.* systematically studied a biologically-relevant SZ candidate gene, *DPYSL2*, and demonstrated a strong association of two sex-specific risk haplotypes. The authors provide strong evidence that SZ-associated *DPYSL2* sequence variants have functional significance, including a 5'-UTR polymorphic DNR. The data suggests that the length of the DNR perturbs a 5'-TOP sequence and influences translation efficiency as regulated by mTOR signaling.
- 73–80 **Mean of the Typical Decoding Rates: A New Translation Efficiency Index Based on the Analysis of Ribosome Profiling Data**
Alexandra Dana and Tamir Tuller
 Translation of mRNA to proteins by ribosomes is a central cellular process, with ramifications related to every biomedical discipline. However, there are currently no approaches that provide genome-wide estimation of translation rates in different cellular conditions. The authors introduce a graphical user interface application (GUI) that provides such estimations; the method is based on a novel filtering of experimental measurements of ribosomal densities which can be very partial and biased. For organisms with ribosome densities data, the GUI can estimate the translation rates of all coding sequences. These estimations correlated well with various direct experimental measurements of translation.
- 81–91 **Cooperation of *DLC1* and *CDK6* Affects Breast Cancer Clinical Outcome**
Xiaofeng Dai, Lu Li, Xiuxia Liu, Weiguo Hu, Yankun Yang, and Zhonghu Bai
 Dai *et al.* report an association between the synergistic effect of *DLC1* and *CDK6* and breast cancer survival. High *DLC1* and low *CDK6* expression is associated with good prognosis. An intronic SNP pair of the two genes was found to cooperatively influence their expression by fitting the over-dominant model. *DLC1* and *CDK6* are proposed to show a dominant negative effect at the translational level, given evidence shown by their relevant proteins. These findings link the germline genetic polymorphisms and synergistic effect of *DLC1* and *CDK6* with breast cancer progression, tailoring the clinical treatments for such patients based on their genetic susceptibility.
- 93–101 **The Histone H3 Lysine 9 Methyltransferase *DIM-5* Modifies Chromatin at *frequency* and Represses Light-Activated Gene Expression**
Catherine E. Ruesch, Mukund Ramakrishnan, Jinhee Park, Na Li, Hin S. Chong, Riasat Zaman, Tammy M. Joska, and William J. Belden
 Ruesch *et al.* document how the role of the histone H3 lysine 9 methyltransferase *DIM-5* functions at the circadian clock gene *frequency* in *Neurospora crassa*. *DIM-5* and H3K9me3 are needed to attenuate light-activated transcription of the *frequency* gene.

- 103–110 **Developmental Analysis of Spliceosomal snRNA Isoform Expression**
Zhipeng Lu and A. Gregory Matera
 Pre-mRNA splicing is a critical step in eukaryotic gene expression that contributes to proteomic, cellular, and developmental complexity. Small nuclear (sn)RNAs are core spliceosomal components; however, the extent to which differential expression of snRNA isoforms regulates splicing is completely unknown. Spliceosomal snRNAs are typically expressed from multi-copy gene families. Lu and Matera performed a comprehensive analysis of snRNA expression patterns in *D. melanogaster* and compared it with other species. The analysis showed that the expression pattern of spliceosomal snRNA isoforms becomes less complex as development progresses, to the point where a single isoform tends to dominate the expression landscape.
- 111–121 **Kar5p Is Required for Multiple Functions in Both Inner and Outer Nuclear Envelope Fusion in *Saccharomyces cerevisiae***
Jason V. Rogers and Mark D. Rose
 When nuclei fuse, both the inner and outer membranes must fuse. Rogers and Rose show that during yeast mating the highly conserved transmembrane protein Kar5p has multiple roles in nuclear fusion including Mps3p-dependent SPB-localization, Prm3p recruitment, and inner nuclear membrane fusion. For inner membrane fusion, Kar5p may physically couple the two nuclear membranes, and/or directly catalyze fusion.
- 123–131 **Identical Substitutions in Magnesium Chelatase Paralogs Result in Chlorophyll-Deficient Soybean Mutants**
Benjamin W. Campbell, Dhananjay Mani, Shaun J. Curtin, Rebecca A. Slattery, Jean-Michel Michno, Donald R. Ort, Philip J. Schaus, Reid G. Palmer, James H. Orf, and Robert M. Stupar
 Gene redundancy is commonly thought to provide a buffer against phenotypic changes when one of the duplicates is mutated. In this study, three missense mutations that cause chlorophyll deficiency phenotypes are cloned from the paleopolyploid soybean genome. These mutations are identified within two paralogous magnesium chelatase genes. The authors demonstrate that nearly identical mutant phenotypes are caused by identical mutations occurring independently in paralogous gene pairs. These data provide evidence that paralogous genes that interact with one another may be more vulnerable to phenotypic alterations. Thus sequence divergence between such paralogs may be constrained.
- 133–144 **High-Resolution Linkage Map and Chromosome-Scale Genome Assembly for Cassava (*Manihot esculenta* Crantz) from 10 Populations**
International Cassava Genetic Map Consortium (ICGMC)
 Cassava (*Manihot esculenta* Crantz) is an important staple crop in Africa, South America, and Asia, and there is a need to improve the crop for disease resistance, increased yield, and other traits. The authors present an integrated genetic map for cassava. They used genotyping-by-sequencing (GBS) to generate SNP-based genetic maps from 10 mapping populations. They combined the maps, recapitulating the 18 linkage groups of cassava, and used the composite map to anchor and orient genome scaffolds representing 91% of the genes. This combined map is an important tool that will underpin future genetic and genomic approaches to improving cassava.

MUTANT SCREEN REPORT

- 145–155 **Parallel Profiling of Fission Yeast Deletion Mutants for Proliferation and for Lifespan During Long-Term Quiescence**
Theodora Sideri, Charalampos Rallis, Danny A. Bitton, Bruno M. Lages, Fang Suo, María Rodríguez-López, Li-Lin Du, and Jürg Bähler
 Sideri *et al.* introduce a new quiescence model to study chronological lifespan in fission yeast. They apply parallel mutant profiling by barcode sequencing to screen pooled deletion mutants to determine which mutants become enriched or under-enriched as a function of age during quiescence. The most long-lived mutants include both known aging genes in other model systems and genes not previously implicated in aging. The authors also apply parallel mutant profiling to compare proliferation under a standard growth condition. These two screens provide a rich resource for further studies, and they suggest that quiescence can provide unique insights into cellular aging.