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April 2017 • VOLUME 7 • ISSUE 4 • www.g3journal.org

MUTANT SCREEN REPORT

1061–1084 Identification of Genes in *Saccharomyces cerevisiae* that Are Haploinsufficient for Overcoming Amino Acid Starvation

Nancy S. Bae, Andrew P. Seberg, Leslie P. Carroll, and Mark J. Swanson

Starvation for amino acids in the yeast *Saccharomyces cerevisiae* induces a cellular response that is conserved in eukaryotes. Through haploinsufficiency screening, we have identified novel genes in the response to starvation, including several genes that have not yet been assigned any function. We have also uncovered a problem with using the heterozygous deletion collection of yeast for some screens. Our data expand upon the knowledge of and associate genes and pathways with the eukaryotic starvation response.

INVESTIGATIONS

1085–1095 A Whole-Transcriptome Approach to Evaluating Reference Genes for Quantitative Gene Expression Studies: A Case Study in *Mimulus*

Kimmy A. Stanton, Patrick P. Edger, Joshua R. Puzey, Taliesin Kinser, Philip Cheng, Daniel M. Vernon, Nancy R. Forsthoefel, and Arielle M. Cooley

Whole transcriptome approaches represent a promising yet underutilized tool for identifying stably expressed reference genes for qPCR. We demonstrate using two *Mimulus* species how RNA-seq can be used to discover and rigorously evaluate reference genes for qPCR, exponentially expanding the pool of candidate genes compared to a gene-by-gene approach. The methods proposed here can be used in any organism, including non-model systems. Our analyses reveal that mean expression is more robust than expression variability, with respect to both environmental and methodological differences, and we discuss the implications of this finding for the experimental design of transcriptome-enabled reference gene discovery.

1097–1108 Triploid Production from Interspecific Crosses of Two Diploid Perennial *Helianthus* with Diploid Cultivated Sunflower (*Helianthus annuus* L.)

Zhao Liu, Gerald J. Seiler, Thomas J. Gulya, Jiujuan Feng, Khalid Y. Rashid, Xiwen Cai, and Chao-Chien Jan

A unique production of triploid hybrids was observed while crossing several wild diploid perennial *Helianthus maximiliani* and *H. nuttallii* populations with cultivated sunflower. The triploid F₁s contain an excess of the paternal genome. A low percentage of unreduced 2n male gametes in the N102 population was observed at meiosis II. The triploids could have resulted from preferential fertilization of the 2n male gametes with the female gametes from cultivated sunflower. This discovery will have significant implications for the evolution of sunflower and other plant species. Understanding the genetic control of this trait will facilitate its utilization in sunflower breeding.

- 1109–1116 **Resistance to Multiple Soil-Borne Pathogens of the Pacific Northwest, USA Is Colocated in a Wheat Recombinant Inbred Line Population**
Alison L. Thompson, Aaron K. Mahoney, Richard W. Smiley, Timothy C. Paulitz, Scot Hulbert, and Kim Garland-Campbell
- Soil-borne pathogens are a major constraint to yield for dryland wheat (*T. aestivum* L.) production in the Pacific Northwest (PNW). Challenges associated with identifying resistant wheat accessions to soil-borne pathogens make these traits ideal candidates for quantitative trait loci (QTL) mapping for marker-assisted breeding strategies. In this study, QTL associated with reduced disease symptomology to four soil-borne pathogens of the PNW were found to be co-localized on the 5A wheat chromosome. This co-localization suggests shared gene(s) contribute to the resistance for multiple soil-borne pathogens and will be useful to wheat breeding programs.
- 1117–1126 **Variants of the Sir4 Coiled-Coil Domain Improve Binding to Sir3 for Heterochromatin Formation in *Saccharomyces cerevisiae***
Anke Samel, Adam Rudner, and Ann E. Ehrenhofer-Murray
- Gene silencing in yeast is mediated by the Silent Information Regulator complex (SIR). Mutations in the AAA+ domain of Sir3 disrupt its interaction with Sir4 and abrogate silencing. Which region of Sir4 interacts with this loop of Sir3? The authors identified that a mutation of threonine 1314 to serine, which removes a methyl group in the Sir4 coiled-coil domain, restored HM silencing, and two additional mutations close-by also restored silencing at the telomeres. Importantly, these mutations also restore the physical interaction between Sir3 and Sir4, thus pinpointing this region of Sir4 as the contact site with the Sir3 AAA+ loop.
- 1127–1136 **Comparative Transcriptomics of Malaria Mosquito Testes: Function, Evolution, and Linkage**
Bryan J. Cassone, Raissa G. G. Kay, Matthew P. Daugherty, and Bradley J. White
- In our study, we performed RNA sequencing of *An. gambiae* and *An. merus* carcass and testes to explore tissue- and species-specific patterns of gene expression. Our data provides strong support for transcriptional repression of X-linked genes in the male germline, which likely drives demasculinization of the X chromosome. Testes-biased genes predominately function in cellular differentiation and show a number of interesting patterns indicative of their rapid evolution, including elevated dN/dS values, low evolutionary conservation, poor annotation in existing reference genomes, and a high likelihood of differential expression between species.
- 1137–1147 **Natural Genetic Variation in the *Caenorhabditis elegans* Response to *Pseudomonas aeruginosa***
Natalia Martin, Jogender Singh, and Alejandro Aballay
- We studied natural variation in the response of *Caenorhabditis elegans* to infection using two *C. elegans* strains that carry the same allele of NPR-1, which is a G-protein-coupled receptor required for defense against pathogen infection. We found that a wild isolate compensates for the deficiency in the NPR-1-controlled responses and determined that the resistance to infection maps to a region on chromosome V. We found that the compensatory mechanism relies exclusively on pathogen avoidance. Our findings underscore the importance of pathogen-specific behavioral defense in the wild, which seems to be favored over the more energy costly activation of cellular defenses.
- 1149–1155 **The Nuclear Cap-Binding Complex Mediates Meiotic Silencing by Unpaired DNA**
Logan M. Decker, Hua Xiao, Erin C. Boone, Michael M. Vierling, Benjamin S. Shanker, Shanika L. Kingston, Shannon F. Boone, Jackson B. Haynes, and Patrick K.T. Shiu
- In the orange bread mold *Neurospora*, genes unpaired during meiosis are targeted by a mechanism known as meiotic silencing by unpaired DNA (MSUD). Many proteins are involved in this process, including common RNAi proteins such as Dicer and Argonaute, which are members of the meiotic silencing complex (MSC) that examine exported RNAs outside of the nuclear pores. In this study, the authors have shown that the well-characterized eukaryotic cap-binding complex (CBC) plays a role in meiotic silencing. CBC interacts with a component (Argonaute) of MSC, directly linking the two previously-unrelated RNA factors.

- 1157–1164 **Genetic Analysis of Teosinte Alleles for Kernel Composition Traits in Maize**
Avinash Karn, Jason D. Gillman, and Sherry A. Flint-Garcia
- Maize was domesticated from its wild ancestor teosinte 9000 years ago in central Mexico, and has been subjected to modern plant breeding over the past 100 years. The domestication and breeding processes resulted in reduced genetic diversity underlying kernel composition traits. We conducted QTL mapping for kernel starch, protein, and oil in a maize-teosinte NIL population. Several of the QTL in our population were previously identified in maize, but we also identified several new grain composition QTL in our population. Ultimately, these novel teosinte alleles can be mined for useful variation to improve corn for producers and consumers.
- 1165–1176 **A Population Genomics Approach to Assessing the Genetic Basis of Within-Host Microevolution Underlying Recurrent Cryptococcal Meningitis Infection**
Johanna Rhodes, Mathew A. Beale, Mathieu Vanhove, Joseph N. Jarvis, Shichina Kannambath, John A. Simpson, Anthea Ryan, Graeme Meintjes, Thomas S. Harrison, Matthew C. Fisher, and Tihana Bicanic
- Whole genome sequencing can be used to illuminate the genetic basis of relapse of fungal meningitis infections in sub-Saharan Africa. Rhodes *et al.* describe the prevalence of recurrent infection by the original infecting isolate, mixed infections and a novel hypermutator infection. These hypermutators contained nonsense mutations in genes encoding DNA mismatch repair proteins, leading to rapid within-host adaptation and evolution of drug resistance.
- 1177–1189 **Construction of a High-Density American Cranberry (*Vaccinium macrocarpon* Ait.) Composite Map Using Genotyping-by-Sequencing for Multi-pedigree Linkage Mapping**
Brandon Schlautman, Giovanny Covarrubias-Pazarán, Luis Diaz-Garcia, Massimo Iorizzo, James Polashock, Edward Grygleski, Nicholi Vorsa, and Juan Zalapa
- A multi-pedigree linkage mapping study was conducted to characterize the cranberry genome and to generate genomic resources for molecular-assisted breeding. In total, 6073 markers, including 5437 single nucleotide polymorphisms (SNPs) generated by genotyping-by-sequencing (GBS) were positioned in a composite map. More than 53% of the SNPs were mapped in two or more of the full-sib populations, suggesting GBS is suitable for discovering and genotyping SNPs that are transferable across cranberry genetic studies. The high-density of mapped SNPs allowed characterization of important chromosome structural aspects including identification of segregation distortion regions, centromere placement, and anchoring of cranberry nuclear scaffolds.
- 1191–1199 **Human Cell Assays for Synthesis-Dependent Strand Annealing and Crossing over During Double-Strand Break Repair**
Grzegorz Zapotoczny and Jeff Sekelsky
- There are numerous pathways for repair of DNA double-strand breaks. Unfortunately, there have been no assays to study a major repair pathway, synthesis-dependent strand annealing, in human cells. We describe such an assay here, and well as a second assay that can specifically measure the rate of crossing over during repair.
- 1201–1209 **Unstable Inheritance of 45S rRNA Genes in *Arabidopsis thaliana***
Fernando A. Rabanal, Viktoria Nizhynska, Terezie Mandáková, Polina Yu. Novikova, Martin A. Lysak, Richard Mott, and Magnus Nordborg
- Genome size in *Arabidopsis thaliana* varies by over 10%, mostly due to variation in 45S rRNA gene copy number. Surprisingly, association mapping does not identify either of the two 45S rRNA gene clusters, suggesting that copy number may be too unstable to be mapped in natural populations. Here we show that both clusters vary greatly, and that although copy number appears to be stable across single generations, changes become apparent within tens of generations. As a consequence, copy number is heritable in pedigrees, but not on population time scales, and represents a *bona fide* case of missing heritability.

- 1211–1214 **Revisiting Suppression of Interspecies Hybrid Male Lethality in *Caenorhabditis* Nematodes**
Lauren E. Ryan and Eric S. Haag
 In a previous issue of G3, Ragavapuram *et al.* (2016) reported that loss of *him-8* in hermaphrodites of the nematode *Caenorhabditis briggsae* could rescue hybrid male sterility in crosses with males from *C. nigoni*. The authors have attempted to replicate this result, using methods that prevent all self-fertilization in *C. briggsae* hermaphrodites. They are unable to reproduce hybrid male rescue, suggesting the previous result was misinterpreted.
- 1215–1223 **An Induced Chromosomal Translocation in Soybean Disrupts a *KASI* Ortholog and Is Associated with a High-Sucrose and Low-Oil Seed Phenotype**
Austin A. Dobbels, Jean-Michel Michno, Benjamin W. Campbell, Kamaldeep S. Viridi, Adrian O. Stec, Gary J. Muehlbauer, Seth L. Naeve, and Robert M. Stupar
 In this study, a soybean mutant with high sucrose and low oil levels in the seed was found to be caused by a unique chromosomal rearrangement caused by fast neutron mutagenesis. Structural and genetic mapping of this mutant revealed an association between the seed phenotype and a reciprocal translocation between chromosomes 8 and 13. This translocation disrupted a single gene on chromosome 8, identified as a *KAS I* ortholog. This work demonstrates the power of mutagenesis and structural genomics to identify unique genetic events and their biological consequences.
- 1225–1237 **Evolution of Gene Expression Balance Among Homeologs of Natural Polyploids**
Jasdeep S. Mutti, Ramanjot K. Bhullar, and Kulvinder S. Gill
 Using 2180 structurally well-characterized genes, the authors have shown that most of the homoeologous copies from the three wheat genomes express from two or more gene copies. Using different analyses, the authors have shown that the evolution of different gene expression control is a significant process creating novel gene functions to shape polyploid evolution. Several interesting observations were obtained from this study such as tissue-specific homoeolog expression and evidence of dosage compensation. In addition, the study also found the effect of chromosomal location on expression of the homoeologs, and changes of DNA methylation due to chromosomal aneuploidy.
- 1239–1249 **The Pivotal Role of Protein Phosphorylation in the Control of Yeast Central Metabolism**
Panayotis Vlastaridis, Athanasios Papakyriakou, Anargyros Chaliotis, Efstratios Stratikos, Stephen G. Oliver, and Grigorios D. Amoutzias
 Protein phosphorylation is the most abundant eukaryotic post-translational modification and acts as a molecular switch or rheostat to control protein function. Thus, the manipulation of protein phosphorylation has great potential for the precise control of specific protein functions. Our computational analyses of phosphoproteomic datasets, together with functional omic and evolutionary data from the best-studied model eukaryote, the yeast *Saccharomyces cerevisiae*, reveal the pivotal role of protein phosphorylation in central metabolism and identify high-quality p-sites. These sites represent potential targets for manipulation to achieve biotechnologically important phenotypes.
- 1251–1257 **Genome-Wide Screen Reveals *sec21* Mutants of *Saccharomyces cerevisiae* Are Methotrexate-Resistant**
Lai H. Wong, Stephane Flibotte, Sunita Sinha, Jennifer Chiang, Guri Giaever, and Corey Nislow
 Drug resistance is a consequence of how most modern medicines work. Drugs exert pressure on cells that causes death or the evolution of resistance. Indeed, highly specific drugs are rendered ineffective by a single DNA mutation. In this study we apply the drug methotrexate, which is widely used in cancer and rheumatoid arthritis, and perform evolution experiments on Baker's yeast to ask the different ways in which cells become drug resistant. Because of the conserved nature of biological pathways between yeast and man, our results can inform how the same mechanism may operate to render human cells resistant to treatment.
- 1259–1265 **Unconventional Recombination in the Mating Type Locus of Heterothallic Apple Canker Pathogen *Valsa mali***
Zhiyuan Yin, Xiwang Ke, Zhengpeng Li, Jiliang Chen, Xiaoning Gao, and Lili Huang
 Sexual reproduction in filamentous ascomycetes is controlled by mating type (*MAT*) locus, including two idiomorphs *MAT1-1* and *MAT1-2*. In heterothallic ascomycete fungi, the *MAT* locus carries either *MAT1-1* or *MAT1-2* idiomorph. Here, we found that the heterothallic apple canker pathogen *Valsa mali* has an unconventional *MAT* locus. Two flanking genes *COX13* and *APN2* were cooperated into *MAT1-1*, and then *MAT1-2* acquired *MAT1-1-2*, *COX13* and *APN2* by unequal recombination, and finally these three genes diverged independently due to different selection pressure. These results provide insight into the evolution of the mating systems in Sordariomycetes.

- 1267–1276 **Genetic Adaptation of Schizothoracine Fish to the Phased Uplifting of the Qinghai–Tibetan Plateau**
Dongsheng Zhang, Mengchao Yu, Peng Hu, Sihua Peng, Yimeng Liu, Weiwen Li, Congcong Wang, Shunping He, Wanying Zhai, Qianghua Xu, and Liangbiao Chen
- Schizothoracine is a subfamily of the Euteleostei family, Cyprinidae, and highly endemic to the Qinghai-Tibetan Plateau (QTP). Previous studies indicated that diversification of the subfamily is closely associated with uplift of QTP but it is short in genetic evidence. In this study, through genome-wide sequence comparison, we elucidated genetic evolution patterns concurrent with the phased uplifting of QTP in the schizothoracines, and revealed divergent adaptive features among lineages of schizothoracines from different altitudes, such as molecular regulations involved in cardiovascular development. The results shed new lights on the mechanisms of genetic adaptation to high-altitude environments in teleosts.
- 1277–1286 **Automated Phenotyping Indicates Pupal Size in *Drosophila* Is a Highly Heritable Trait with an Apparent Polygenic Basis**
R. Guy Reeves and Diethard Tautz
- The study of human height has much advanced our understanding of highly complex phenotypes. We detail the properties of a previously unexplored trait in *Drosophila melanogaster* that shares many salient properties with human height. We demonstrate that pupal case length is among the most heritable traits reported. Described is a simple phenotyping system with which a single operator can score >5000 individuals per day. A scan in 195 recombinant inbred lines did not reveal a major effect locus, compatible with the assumption that pupal size in *Drosophila* could be a similarly complex trait as height in humans.
- 1287–1299 **Independent Maternal and Fetal Genetic Effects on Midgestational Circulating Levels of Environmental Pollutants**
Michela Traglia, Lisa A. Croen, Kristen Lyall, Gayle C. Windham, Marty Kharrazi, Gerald N. DeLorenze, Anthony R. Torres, and Lauren A. Weiss
- Maternal levels of environmental pollutants have shown significant, but negative associations with offspring ASD outcome. We report that maternal mid-pregnancy serum levels of pollutants showed high maternal and fetal heritability. Significant maternal loci were located in the *CYP2B6* gene and near the *SH3GL2* gene, involved in xenobiotic and lipid metabolism. Fetal genetic loci associated with maternal levels of congeners were detected near the potential metabolic genes *LOXHD1* and *PTPRD*, implicated in neurodevelopment. Our results support strong genetic control of mid-gestational biomarkers of environmental exposures by non-overlapping maternal and fetal loci. We speculate that fetal genes expressed in placenta can influence maternal physiology and the transplacental transfer of pollutants.
- 1301–1314 **Genomic Data Quality Impacts Automated Detection of Lateral Gene Transfer in Fungi**
Pierre-Yves Dupont and Murray P. Cox
- Lateral gene transfer (LGT), a method by which genes move between species, is increasingly a default explanation for genes displaying unexpected composition or phylogeny. LGT is common and evolutionarily influential in bacteria. However, detection tools, many originally built to detect bacterial LGTs, are being applied to eukaryotes, leading to a growing compendium of putative eukaryotic LGTs. This study assesses the suitability of these methods for detecting LGT in fungal genomes. We show that they exhibit low power to identify LGTs in uncurated datasets, which are commonly used by researchers, thus questioning many of the LGTs proposed in the fungal literature.
- 1315–1321 **A Nonsense Variant in the *ST14* Gene in Akhal-Teke Horses with Naked Foal Syndrome**
Anina Bauer, Theresa Hiemesch, Vidhya Jagannathan, Markus Neuditschko, Iris Bachmann, Stefan Rieder, Sofia Mikko, M. Cecilia Penedo, Nadja Tarasova, Martina Vitková, Nicolò Sirtori, Paola Roccabianca, Tosso Leeb, and Monika M. Welle
- This study identified a likely candidate causative variant for naked foal syndrome (NFS) in Akhal-Teke horses. The variant introduces a premature stop codon into the *ST14* gene. Comparable *ST14* genetic variants cause autosomal recessive congenital ichthyosis 11 in humans.

- 1323–1337 **Planar Cell Polarity Effector Fritz Interacts with Dishevelled and Has Multiple Functions in Regulating PCP**
Ying Wang, Victor F. Naturale, and Paul N. Adler
- The Planar cell Polarity Effector (PPE) genes *inturned*, *fuzzy* and *fritz* are downstream components in the *frizzled/starry night* signaling pathway. The protein products of these genes all accumulate asymmetrically in epidermal cells and this requires the function of the upstream genes. We report here that two of the PPE proteins interact directly with the upstream Dsh protein. The three PPE proteins have been thought to function as a unit but we found that both Frtz and Fy can impact PCP in the complete absence of In. In vivo imaging shows that Frtz distribution is dynamic.
- 1339–1347 **Genetic and Transgenic Reagents for *Drosophila simulans*, *D. mauritiana*, *D. yakuba*, *D. santomea*, and *D. virilis***
David L. Stern, Justin Crocker, Yun Ding, Nicolas Frankel, Gretchen Kappes, Elizabeth Kim, Ryan Kuzmickas, Andrew Lemire, Joshua D. Mast, and Serge Picard
- Species of the *Drosophila melanogaster* species subgroup, including the species *D. simulans*, *D. mauritiana*, *D. yakuba*, and *D. santomea*, have long served as model systems for studying evolution. Studies in these species have been limited, however, by a paucity of genetic and transgenic reagents. Here we described the construction and characterization of new tools that will allow high-resolution genetic studies and that will simplify transgenic assays. These reagents provide an experimental platform for accelerating research on the evolution of genes, genomes and phenotypes.
- 1349–1356 **Time-Variant Genetic Effects as a Cause for Preterm Birth: Insights from a Population of Maternal Cousins in Sweden**
Julius Juodakis, Jonas Bacelis, Ge Zhang, Louis J. Muglia, and Bo Jacobsson
- Preterm delivery is the leading cause of neonatal mortality worldwide, yet its etiology remains largely unexplained. We propose that genetic factors controlling this trait could act in a non-uniform manner during pregnancy, with each factor having a ‘window of sensitivity’. We test this hypothesis by modeling clinical data from maternal cousins in Sweden, and observe best fit when most factors had time-variant effects. We believe that the tools and concepts presented here should prove useful for the design of future studies and provide new insights into the genetic architecture determining human gestational age.
- 1357–1363 ***pnp4a* Is the Causal Gene of the Medaka Iridophore Mutant *guanineless***
Tetsuaki Kimura, Yusuke Takehana, and Kiyoshi Naruse
- The causal gene of the iridophore-less mutant is the last piece of the jigsaw for making see-through medaka by genome editing. The medaka has four different pigment cells: black melanophores, yellow xanthophores, white leucophores, and silver iridophores. To date, the causal genes of melanophore, xanthophore, and leucophore mutants have been identified, but the causal gene for the iridophore-less mutant remains unknown. Additionally, the causal gene of roy orbison, which is responsible for the iridophore-less phenotype in the casper fish, is currently unknown. We describe the iridophore mutant, *guanineless* (*gu*), and showed that the causal gene of *gu* is *pnp4a*.
- 1365–1376 **A Dense Brown Trout (*Salmo trutta*) Linkage Map Reveals Recent Chromosomal Rearrangements in the *Salmo* Genus and the Impact of Selection on Linked Neutral Diversity**
Maeva Leitwein, Bruno Guinand, Juliette Pouzadoux, Erick Desmarais, Patrick Berrebi, and Pierre-Alexandre Gagnaire
- We provide a high density sex- and lineage-averaged brown trout (*Salmo trutta*) linkage map based on 3977 markers ordered in 40 linkage groups. Comparison with Atlantic salmon (*S. salar*) revealed that most chromosomal rearrangements occurred within the *S. salar* branch, whereas the brown trout remained closer to the ancestral state. We estimated the local recombination rate in brown trout and found a significant positive correlation with genome-wide nucleotide diversity, indicating that selection constrains variation at linked neutral sites in brown trout. This linkage map represents an unprecedented genomic resource for this species.
- 1377–1383 **Genotype Imputation To Improve the Cost-Efficiency of Genomic Selection in Farmed Atlantic Salmon**
Hsin-Yuan Tsai, Oswald Matika, Stefan McKinnon Edwards, Roberto Antolín-Sánchez, Alastair Hamilton, Derrick R. Guy, Alan E. Tinch, Karim Gharbi, Michael J. Stear, John B. Taggart, James E. Bron, John M. Hickey, and Ross D. Houston
- This article describes the use of genotype imputation as part of a cost-effective genotyping strategy for genomic selection in salmon breeding.