

Future Functions for Fungal Genes of Unknown Function

Alexander Idnurm^{1*} and Robert A. Cramer²

¹Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, USA

²Department of Microbiology and Immunology, Geisel School of Medicine, Dartmouth College, Hanover, USA

Editorial

The genomics revolution continues to increase our information on the gene content in fungal species. However, few of these genes have been examined experimentally, and in many cases up to 50% of fungal genes have no predictable function. A clear direction in utilizing the incredible genomics data sets is to define the functions of these genes in fungi. This is not a trivial task, as anyone who has unsuccessfully sought the function for just one gene can attest. Moreover, requesting funding for the study of a gene of unknown function is typically associated with low enthusiasm on grant proposals. Yet, a full understanding of gene function in fungal genomes is potential key for fully harnessing the beneficial uses of fungi to humanity and for reducing the incidence of fungal disease. Importantly, the term “function” can have different interpretations. For instance, a biochemist’s perspective would include an enzymatic activity for the gene product, while a geneticist’s perspective would require a phenotype for a strain carrying a mutation in the gene. Regardless of definition, it seems highly probable that significant advances in our understanding of fungal biology will come from study of currently unknown function genes and their products.

Gene function, based on the phenotypes of mutants, has already been addressed on a large scale in the budding yeast *Saccharomyces cerevisiae*, in which deletion sets have been created in multiple strain backgrounds [1,2], and in the fission yeast *Schizosaccharomyces pombe* [3]. Related projects with ongoing support from the US National Institutes of Health include generating sets of gene deletion strains for the filamentous ascomycete *Neurospora crassa* and the basidiomycete yeast *Cryptococcus neoformans* [4,5]. The omic scale studies in *S. cerevisiae* have deleted all the genes, all proteins were localized in the cell with GFP-fusions, and transcript levels analyzed under myriad growth conditions. Yet until recently, about 80% of the *S. cerevisiae* mutants had no reported phenotype.

Two explanations are commonly given for lack of phenotypes in gene deletion strains. One is redundancy in gene function, through cases of gene duplication or multiple gene family members, or potential undefined compensatory mechanisms. Studies describing a fungal genome commonly analyze gene abundance, as homologs or classified in different functional categories, with the implication that gene number expansion reflects a unique or important aspect about their lifecycle. For example, compared to other fungi the wood rotting species have more lignin-degrading peroxidases [6], plant pathogens have more enzymes to break down pectin and cellulose [7], and the dermatophytes have more chitin-binding proteins and proteases [8]. These correlations are difficult to test by mutating individual genes if they have redundant function. Furthermore, and in argument against redundancy, there are examples of strains carrying deletions of pairs of related genes that have no additive effect beyond the single mutant strain [9]. The second explanation for a gene deletion strain with no phenotype is a lack of knowledge about the function of the gene of interest that makes selecting environmental conditions to screen problematic. For genes for unknown function, it may just be that the relevant environment or stress has not yet been tested or identified.

An alternative hypothesis is that every gene in the genome has

a specific, non-redundant function. A key study in support of this hypothesis took a chemical genomics approach, by examining the growth of the yeast deletion set strains in parallel in response to numerous chemicals [10]. This study defined fitness differences for 97% of the *S. cerevisiae* genes in the genome, and those remaining 3% without a function are dubious genes. This would support the concept that all genes in fungi have a function, none are redundant (even in an organism like *S. cerevisiae* characterized by its ancestral whole genome duplication event), and one simply has to search harder to find phenotypes to infer function.

However, this returns to the definition of function. Is it a reduced fitness of a deletion strain within a pool of thousands of other strains, to a chemical or chemical class to which the species may never have been exposed? Another hypothesis is consistent with answering this question. Some genes do not have a current function and yet are not lost by mutation. Being under neutral selection would provide an advantage to evolve in which natural selection could act on these genes if environmental conditions change in the future. One would then predict that species living under variable conditions should maintain a reservoir of unused “non-functional” genes. Those species would be fitter in the future when subsequent generations experience new conditions in which these genes could be advantageous. Along these lines, it seems important to recognize that our sequencing projects and indeed our functional analyses of fungal genes and genomes is taking place in a minute time period of fungal evolution. We are simply glimpsing a snapshot in a moment in time, though we have in hand the tools and technologies to ask what this means both in the present and in the future of gene function.

Testing this hypothesis is likely best addressed through *in vitro* evolution experiments, in which selective pressure can be applied or removed and the newly evolved strains examined at the genomics level, starting with genome resequencing technology to determine if new functions have arisen for previously “non-functional” genes. A comparison between free-living and host-associated species, i.e., species subject to more or less variation during their evolution, would also be highly informative. It is worthy to note that while many initial genome sequencing projects for fungi of interest focused on commonly-utilized laboratory strains, advances in sequencing technologies are encouraging broader scope sequencing projects of environmental isolates that should encourage gene function and comparative genomics studies

***Corresponding author:** Alexander Idnurm, Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri-Kansas City, Kansas City, USA, E-mail: idnurma@umkc.edu

Received January 18, 2013; **Accepted** January 20, 2013; **Published** January 27, 2013

Citation: Idnurm A, Cramer RA (2013) Future Functions for Fungal Genes of Unknown Function. Fungal Genom Biol 3: e112. doi:10.4172/2165-8056.1000e112

Copyright: © 2013 Idnurm A, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

with the laboratory strains. This is of particular relevance for species that can carry dispensable chromosomes that may vary in number or presence between strains [7,11], and whose genes may be missed in the sequence of a single laboratory strain.

The next exciting step in genomics is the task to assign function to the elements encoded within the DNA of a species. This is daunting because of the personnel and financial resources required; e.g. see commentaries on the costs of the ENCODE project for the human genome [12,13]. These genome-wide initiatives must be encouraged. Further generation of whole genome deletion collections in fungi of interest would provide strong community resources to define fungal gene function. At the same time less criticism should be placed on studies that characterize genes that appear to play a minor role in the biology of the organism. For functional genomics another approach is the return to traditional forward genetics experiments, which start with biology of interest and subsequently track down its genetic basis. These efforts can be aided by the resource of available genome sequences. In summary, now is the time for advancing functional genomics in the fungi, and for genes without functions that will inevitably arise from these experiments it may be unnecessary to worry about what the future may hold for them.

Acknowledgements

Research in the authors' laboratories is supported by NIH-NIAID grants AI081838 and AI094364.

References

1. Giaever G, Chu AM, Ni L, Connelly C, Riles L, et al. (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* 418: 387-391.
2. Dowell RD, Ryan O, Jansen A, Cheung D, Agarwala S, et al. (2010) Genotype to phenotype: a complex problem. *Science* 328: 469.
3. Kim DU, Hayles J, Kim D, Wood V, Park HO, et al. (2010) Analysis of a genome-wide set of gene deletions in the fission yeast *Schizosaccharomyces pombe*. *Nat Biotechnol* 28: 617-623.
4. Colot HV, Park G, Turner GE, Ringelberg C, Crew CM, et al. (2006) A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc Natl Acad Sci USA* 103: 10352-10357.
5. Liu OW, Chun CD, Chow ED, Chen C, Madhani HD, et al. (2008) Systematic genetic analysis of virulence in the human fungal pathogen *Cryptococcus neoformans*. *Cell* 135: 174-188.
6. Floudas D, Binder M, Riley R, Barry K, Blanchette RA, et al. The Paleozoic origin of enzymatic lignin decomposition reconstructed from 31 fungal genomes. *Science* 336: 1715-1719.
7. Ohm RA, Feau N, Henrissat B, Schoch CL, Horwitz BA, et al. (2012) Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen *dothideomycetes* fungi. *PLoS Pathog* 8: e1003037.
8. Martinez DA, Oliver BG, Gräser Y, Goldberg JM, Li W, et al. (2012) Comparative genome analysis of *Trichophyton rubrum* and related dermatophytes reveals candidate genes involved in infection. *MBio* 3: e00259-00212.
9. Dolinski K, Muir S, Cardenas M, Heitman J (1997) All cyclophilins and FK506 binding proteins are, individually and collectively, dispensable for viability in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 94: 13093-13098.
10. Hillenmeyer ME, Fung E, Wildenhain J, Pierce SE, Hoon S, et al. (2008) The chemical genomic portrait of yeast: uncovering a phenotype for all genes. *Science* 320: 362-365.
11. Ma LJ, van der Does HC, Borkovich KA, Coleman JJ, Daboussi MJ, et al. (2010) Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. *Nature* 464: 367-373.
12. Pennisi E (2012) ENCODE project writes eulogy for junk DNA. *Science* 337: 1159-1161.
13. Maher B (2012) ENCODE: The human encyclopaedia. *Nature* 489: 46-48.