



ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

 Current Opinion in
**Genetics
& Development**

Contemporary, yeast-based approaches to understanding human genetic variation

Maitreya J Dunham and Douglas M Fowler

Determining how genetic variation contributes to human health and disease is a critical challenge. As one of the most genetically tractable model organisms, yeast has played a central role in meeting this challenge. The advent of new technologies, including high-throughput DNA sequencing and synthesis, proteomics, and computational methods, has vastly increased the power of yeast-based approaches to determine the consequences of human genetic variation. Recent successes include systematic exploration of the effects of gene dosage, large-scale analysis of the effect of coding variation on gene function, and the use of humanized yeast to model disease. By virtue of its manipulability, small genome size, and genetic tractability, yeast is poised to help us understand human genetic variation.

Addresses

Department of Genome Sciences, University of Washington, Foegen Building, Box 355065, 3720 15th Avenue NE, Seattle, WA 98195-5065, USA

Corresponding authors: Dunham, Maitreya J (maitreya@uw.edu) and Fowler, Douglas M (dfowler@uw.edu)

Current Opinion in Genetics & Development 2013, **23**:xx–yy

This review comes from a themed issue on **Genetics of system biology**

Edited by **Shamil Sunyaev** and **Fritz Roth**

0959-437/\$ – see front matter, © 2013 Elsevier Ltd. All rights reserved.

<http://dx.doi.org/10.1016/j.gde.2013.10.001>

Introduction

With acceleration of sequencing technologies, many human genomes are becoming available from patients, tumors, and thousands of individuals from diverse populations. In parallel, linkage mapping, genome-wide association strategies, and analyses of *de novo* mutations are rapidly linking genomic regions to phenotypes including disease susceptibility. However, defining which genetic variants are causative for phenotype has become rate-limiting. Furthermore, the abundance of rare variation means that sequencing more genomes is unlikely to solve this problem (e.g. [1,2]). We propose that new technologies such as high-throughput DNA sequencing, proteomics, and computational approaches can empower model organism genetics to fill this gap by enabling high-throughput, generic, genome-scale functional assays for characterizing variation in the human genome. Yeast, especially the budding yeast *S. cerevisiae*, is uniquely suited to this task because of its versatility, small genome

size, and powerful array of existing tools (reviewed in [3]). Methods for understanding the consequences of human variation using yeast fall into three broad categories: 1. systematic analysis of gene dosage; 2. recreation of human variants in their yeast orthologs; and 3. cross-species complementation and heterologous expression. In addition to enabling direct measurement of the consequences of specific genetic variants, work in yeast and other model organisms will be necessary for understanding the essential underlying biology. These larger biological questions include the distribution of effect sizes of genetic variants, the contribution of genetic modifiers and the role of epistasis more generally, and, of course, the fundamental molecular mechanisms by which genes and their variants act.

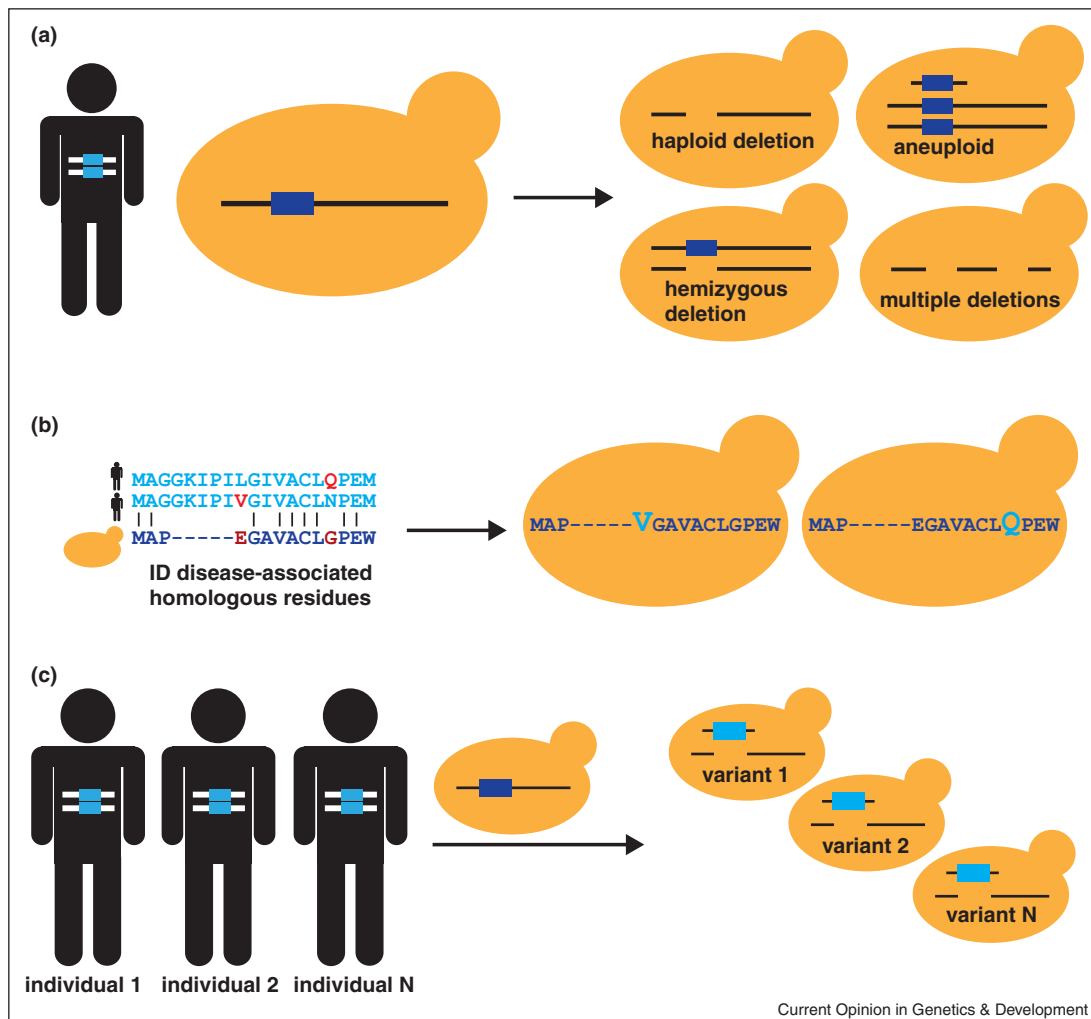
Systematic analysis of gene dosage

Yeast is easily amenable to purposeful manipulation of gene dosage, most frequently via loss of function but increasingly by overexpression as well. Examining the resulting phenotypes can reveal the function of the element whose dosage is changed (Figure 1a). When specific phenotypes are shared, connections between yeast and human can be relatively easy to recognize. Famously, work in yeast correctly predicted the role of the human mismatch repair genes *hPMS1*, *hMLH1*, and *hMSH2* in hereditary non-polyposis colon cancer based on the yeast knockouts' mutator phenotypes [4]. More systematic approaches have now become possible (reviewed in [5]); for example, yeast genes involved in mitochondrial biology were used to identify human orthologs with similar cellular roles [6]. These early studies highlighted the power of gene deletion to make inferences about protein function.

Large-scale studies of the consequences of gene dosage changes are pushing this approach towards its logical conclusion in many organisms. In yeast, a variety of tools are available including comprehensive collections of deletions [7,8], overexpression plasmids [9,10], and hypomorphic alleles [11,12]. These resources have been used effectively to infer the function of previously unannotated genes [13], understand how human drugs interact with alterations in gene dosage [14], and determine how gene loss relates to basic cellular processes such as meiosis [15]. These are a small sample of the hundreds of examples that have been successful so far. Thanks in no small part to these model organism studies, we now have a relatively clear idea of how loss of specific gene functions can result in disease, and some idea of the

2 Genetics of system biology

Figure 1



Methods of assessing human variation in yeast. **(a)** A human gene of interest (light blue) and its orthologous yeast gene (dark blue) are shown. Systematic adjustment of the dosage of the yeast gene (e.g. by deletion or overexpression) followed by examination of the resulting yeast phenotype can reveal the function of the human ortholog. Epistatic interactions can be explored by examining the consequences of multiple deletions. Adding or removing adjacent chromosome segments can reveal the consequences of more complex changes in copy number. **(b)** An alignment between a human gene (light blue) and its yeast ortholog (dark blue) is shown. Variants of interest in the human gene are shown in light red. The sequence alignment is used to identify orthologous positions in the yeast gene (dark red). The human variants of interest are introduced into the yeast ortholog and the consequences for protein function quantified. **(c)** A gene of interest in humans (light blue) and yeast (dark blue) is knocked out in yeast. Each human variant is tested for its ability to complement the yeast deletion.

characteristics of genes which make them more or less likely to be associated with strong phenotypes. For example, for some genes, lack of an obvious knockout phenotype is explained by the presence of a paralog that compensates for the function of the deleted gene, a hypothesis proven at genome scale using double knockout libraries of duplicate genes [16,17]. The effects of heterozygosity have also been explored systematically in yeast; specifically the entire set of haploinsufficient genes is known in both rich media [18] and in many specific conditions (e.g. [19]), giving predictions that may be important for understanding dominance patterns in

human disease alleles. ‘Haploproficient’ genes, those for which heterozygous loss-of-function variants actually improve growth, are predictive of potential driver genes in cancer [20].

In addition to simple cases where the dosage of a single gene is altered, yeast is uniquely suited to the study of more complex alterations in gene dosage. For example, a complete survey of all pairwise gene deletions in yeast is nearing completion, and has already led to a comprehensive understanding of the range of interaction effects between loss-of-function alleles (e.g. [21]). Lethal

pairwise deletions suggest opportunities for genotype-targeted cancer therapeutics: if a tumor harbors a particular mutation, drugs could be used to target a known synthetic lethal interaction partner. Systematic studies in yeast of genes underlying tumor phenotypes such as chromosomal instability have suggested exactly such attractive drug targets [22,23*]. Complex haploinsufficiency, in which heterozygosity at two loci leads to a growth defect, extends the space of potential genetic interactions, with obvious relevance for diploid genetics [24–27].

Events affecting larger numbers of genes simultaneously, such as deletion or amplification of chromosomes or portions thereof, underlie a number of human diseases, most notably cancer, developmental disorders, and a growing variety of neurological diseases (reviewed in [28]). These structural alterations are difficult to model from the data we already have regarding the effects of altering the dosage of single genes because changing the dosage of multiple genes often has unpredictable results. Even simple questions, such as the number and identity of driver genes that contribute to the phenotypic consequences of a particular structural variant, have only been dissected in very specific examples. Study of structural variation in model organisms can elucidate the mechanisms by which these genomic alterations influence human disease and other phenotypes (reviewed in [29]). Yeast is particularly amenable to chromosome engineering approaches to studying these complex events, using classical methods such as chromosome fragmentation vectors, yeast artificial chromosomes, and movement of whole chromosomes using karyogamy deficient mutants, as well as more modern synthetic biology methods. For example, synthesis of an arm of yeast chromosome IX enabled the systematic incorporation of 43 loxP sites [30]. Recombination amongst these sites upon Cre exposure generated a host of rearrangements, insertions, and deletions, whose effects could then be measured in parallel. Thus, synthetic biology approaches in yeast have the potential to enable large-scale studies of the consequences of complex genomic alterations. The data derived from these studies could move us closer to our ultimate goal: a model for predicting the effects of genomic alterations in humans.

Recreation of genetic variants in orthologs

Complete loss-of-function alleles comprise a minority of the relevant genetic variation in humans and other organisms. Most genes have many alleles ranging from complete loss of function to subtle alterations in function. For genomic regions with significant conservation, human variants can be tested for function by making homologous mutations in their yeast orthologs (Figure 1b). For example, *MSH2* alleles associated with hereditary colon cancer were systematically evaluated in yeast, where different alleles were shown to interfere with different

aspects of the protein function [31]. In another success story, variants in *MTO1* discovered by exome sequencing in patients with hypertrophic cardiomyopathy were validated in yeast by recreating the orthologous mutations [32*].

Advances that combine high-throughput DNA sequencing with selection for function have supercharged these approaches by enabling the simultaneous characterization of all possible single amino acid changes in a protein of interest [33]. For example, simultaneous characterization of all possible point mutations in ubiquitin, associated with numerous diseases, identified mutation-sensitive regions and binding partner locations [34*]. These approaches could help narrow down protein regions where pathogenic variants reside, and could contribute to dissecting separation of function, in which variants in the same gene are pathogenic but for different molecular reasons.

Cross-species complementation and heterologous expression

Despite the value of using orthologous genes, conservation-based inference of mutation effects can be fraught [35]. In some cases, direct testing of variants in their native gene context might be more desirable. In yeast, this has been attempted in two ways: cross-species complementation and heterologous expression. Cross-species complementation is simply the ability of human genes to rescue an orthologous loss-of-function mutation in another organism (Figure 1c). The conservation of core cell biology among all organisms means that this approach is often successful, even in simple eukaryotes such as yeast (reviewed in [36–38]). Complementation of yeast mutations by human genes is a classic method that has led to many breakthroughs in the understanding of human gene function (reviewed by [39]). According to the Saccharomyces Genome Database and the Princeton Protein Orthology Database [40], as of 2009, over 350 such experiments had been published, with at least partial complementation reported for over 200 genes. In one study, 25% of yeast essential gene deletions could be rescued by a human sequence [41]. Human gene expression in yeast must be approached with care, however; toxicity from overexpression has been observed in as many as 30% of attempts [42], and partial fusions with the yeast gene are sometimes required for full activity.

One early application of this approach was with cystathione β -synthase (encoded by *CBS* in human and *CYS4* in yeast), where mutations cause homocystinuria. The human *CBS* gene complements *cys4* mutations in yeast [43], and human disease alleles have been recapitulated in yeast [44]. This system has recently been scaled up to test dozens of human alleles for function and cofactor dependencies [45*]. These experiments emphasize another benefit of working with yeast: environments

4 Genetics of system biology

and genetic backgrounds can easily be modified to determine what may exacerbate or relieve the effects of variants, pointing towards potential treatments.

Despite the anecdotal success of cross-species complementation and the development of humanized yeast as models for studies on Parkinson's and apoptosis [46,47], systematic approaches have only recently been made practical by advances in clone libraries and vector engineering. For example, the human ORFeome collection is an ongoing project that together with the Mammalian Gene Collection has assembled a clone library of sequence-confirmed human cDNAs for over 90% of genes, including a growing assortment of splice variants [48,49]. These are available in the Gateway vector system, facilitating their transfer to yeast expression vectors. Collections of humanized yeast strains, generated from these clone collections in combination with the deletion collection, are likely to become a new resource that could be used to easily and rapidly examine variants in many human genes of interest.

Even when a human gene does not have a clear ortholog in yeast, its function may still be studied via heterologous expression. For example, p53, a critical tumor suppressor gene, has no yeast ortholog. Nevertheless, p53 transactivation assays in yeast have been used to study all 2314 point mutations [50] and to identify second-site suppressor mutations [51,52]. Yeast-based measures of variant p53 transactivation have also been useful in teasing apart transactivation-dependent and transactivation-independent p53 activities [53]. In another example, screening of RNA-binding proteins for aggregation in yeast enabled the identification of new amyotrophic lateral sclerosis candidate genes [54**]. Additionally, yeast is an excellent platform for studying protein-protein interactions, both of endogenous and human proteins [55–57]. For example, a two-hybrid approach was used to understand how mutations in *FIG4* cause Charcot-Marie-Tooth disease [58]. Yeast-based complementation or heterologous expression assays could also be coupled to high throughput mutagenesis and deep sequencing-based approaches as described above. These approaches yield a complete sequence-function map for a given gene, revealing how variation in the gene impacts function.

Limitations of model organism approaches

Of course, all model systems have their downsides. Approaches in yeast might be most productively viewed as a method for intelligently prioritizing experiments to be done in more complicated and expensive mammalian models. An obvious caveat of evaluating human alleles in other organisms is that some genes will not be equivalently functional outside their native context of a human cell. Furthermore, some variants may disrupt interactions with proteins not present or too diverged in other systems, or may affect processes such as transcript splicing that are

not recreated in cDNA-based systems. Most problematically, certain pathways including those related to development and multicellularity might be largely off limits in single-celled organisms such as yeast. However, many of these genes have some conserved function in yeast as most genes ultimately function in individual cells.

Surprisingly, these limitations are not as strict as one might suppose because even when the exact biological process is absent, the underlying genetic architecture is often conserved. This fundamental unity of evolution, in which genes and modules are reused for various purposes in different organisms, is detectable by looking at orthologous phenotypes. Two phenotypes are orthologous (so-called 'phenologs' [59*,60]) when they are produced by mutations in a set of common orthologous genes. By taking advantage of phenologs, experimenters can derive nonobvious models for complex human disease in organisms like yeast. For example, a systematic hunt for orthologous human disease phenotypes in model organisms suggested a yeast model for angiogenesis defects, among other potential applications [60]. Thus, phenologs provide a Rosetta stone for those wishing to use model organisms to study the effect of genomic variation on human disease.

Of course, another requirement for these approaches is the presence of a measurable phenotype. The experimenter has some flexibility, as phenotype can be defined as broadly as survival or as specifically as binding to a given target, depending on how the assay is designed. Additionally, the magnitude of the phenotype required has been steadily shrinking and the requirement for a characteristic phenotype is not absolute. Pooled, competition-based schemes can detect small, quantitative changes in function. Rather than relying on a particular, pre-selected phenotype to infer the consequences of variation at a disease-related locus, unbiased phenotyping methods can be used. Examples include high-content screening, in which changes in morphology are measured; proteomics, in which protein abundance and post-translational modification are measured; and RNAseq, in which global changes in the transcriptome are measured. In each case, the effect of disease-associated variants can be inferred by examining global changes (e.g. in gene expression or cellular phenotype) rather than specific ones.

Conclusion

We have argued that yeast is an ideal model organism in which to address the consequences of human genomic variation. We have reviewed the key approaches that have already allowed huge progress in understanding human and yeast genetic perturbations, ranging from single point mutations to entire extra chromosomes. Additionally, the genetics underlying disease is sometimes so poorly understood that model organisms are needed just to define

these basics (reviewed in [61]). For example, complex traits are notoriously difficult to dissect using genomic data. Despite genotyping tens of thousands of individuals, genome wide association studies often report a large amount of missing heritability. Missing heritability could arise from genetic interactions, a possibility recently receiving more attention in the human genetics community [62]. Yeast enable specific crosses to be carried out and the progeny phenotyped quantitatively, shedding light on this central problem [63,64**]. The effects of genetic background also constitute a significant confounding factor in our ability to determine how variation at a given locus impacts phenotype. For example, even gene essentiality can be profoundly different between genetically diverse strain backgrounds [65]. More dense phenotyping could also be helpful, as attempted in a recent 'phenomics' analysis of a collection of diverse yeast strains [66]. Although these approaches remain challenging, yeast offers our best hope for beginning to disentangle these confounding phenomena.

Even more fundamental questions remain, and are central to disease. How many mutational paths are there to a given phenotype? How do complex phenotypes arise? How do mutations at different loci interact? Human genetics approaches to answering these questions are hampered by the complexity of the human genome and our lack of ability to manipulate it. Yeast, free from these limitations, is the perfect system in which to begin to answer these questions.

Acknowledgements

MD is a Rita Allen Foundation Scholar and a Canadian Institute for Advanced Research Fellow. She is supported by grants from the National Institute of General Medical Sciences (P41 GM103533, R01GM094306 and R01GM101091) from the National Institutes of Health.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Nelson MR, Wegmann D, Ehm MG, Kessner D, St Jean P, Verzilli C, Shen J, Tang Z, Bacanu S-A, Fraser D *et al.*: **An abundance of rare functional variants in 202 drug target genes sequenced in 14,002 people.** *Science* 2012, **337**:100-104.
2. Tennessen JA, Bigham AW, O'Connor TD, Fu W, Kenny EE, Gravel S, McGee S, Do R, Liu X, Jun G *et al.*: **Evolution and functional impact of rare coding variation from deep sequencing of human exomes.** *Science* 2012, **337**:64-69.
3. Botstein D, Fink GR: **Yeast: an experimental organism for 21st Century biology.** *Genetics* 2011, **189**:695-704.
4. Strand M, Prolla T, Liskay R, Petes T: **Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair.** *Nature* 1993, **365**:274-276.
5. Perocchi F, Mancera E, Steinmetz LM: **Systematic screens for human disease genes, from yeast to human and back.** *Mol Biosyst* 2008, **4**:18-29.
6. Steinmetz L, Scharfe C, Deutschbauer A, Mokranjac D, Herman Z, Jones T, Chu A, Giaever G, Prokisch H, Oefner P *et al.*: **Systematic screen for human disease genes in yeast.** *Nat Genet* 2002, **31**:400-404.
7. Giaever G, Chu AM, Ni L, Connelly C, Riles L, Véronneau S, Dow S, Lucanu-Danila A, Anderson K, André B *et al.*: **Functional profiling of the *Saccharomyces cerevisiae* genome.** *Nature* 2002, **418**:387-391.
8. Winzler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, Andre B, Bangham R, Benito R, Boeke JD, Bussey H *et al.*: **Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis.** *Science* 1999, **285**:901-906.
9. Ho CH, Magtanong L, Barker SL, Gresham D, Nishimura S, Natarajan P, Koh JLY, Porter J, Gray CA, Andersen RJ *et al.*: **A molecular barcoded yeast ORF library enables mode-of-action analysis of bioactive compounds.** *Nat Biotechnol* 2009, **27**:369-377.
10. Sopko R, Huang D, Preston N, Chua G, Papp B, Kafadar K, Snyder M, Oliver S, Cyert M, Hughes T *et al.*: **Mapping pathways and phenotypes by systematic gene overexpression.** *Mol Cell* 2006, **21**:319-330.
11. Mnaimneh S, Davierwala AP, Haynes J, Moffat J, Peng W-T, Zhang W, Yang X, Pootoolal J, Chua G, Lopez A *et al.*: **Exploration of essential gene functions via titratable promoter alleles.** *Cell* 2004, **118**:31-44.
12. Ben-Aroya S, Coombes C, Kwok T, O'Donnell KA, Boeke JD, Hieter P: **Toward a comprehensive temperature-sensitive mutant repository of the essential genes of *Saccharomyces cerevisiae*.** *Mol Cell* 2008, **30**:248-258.
13. Hess DC, Myers CL, Huttenhower C, Hibbs MA, Hayes AP, Paw J, Clore JJ, Mendoza RM, Luis BS, Nislow C *et al.*: **Computationally driven, quantitative experiments discover genes required for mitochondrial biogenesis.** *PLoS Genet* 2009, **5**:e1000407.
14. Hillenmeyer ME, Fung E, Wildenhain J, Pierce SE, Hoon S, Lee W, Proctor M, St Onge RP, Tyers M, Koller D *et al.*: **The chemical genomic portrait of yeast: uncovering a phenotype for all genes.** *Science* 2008, **320**:362-365.
15. Deutschbauer A, Williams R, Chu A, Davis R: **Parallel phenotypic analysis of sporulation and postgermination growth in *Saccharomyces cerevisiae*.** *Proc Natl Acad Sci USA* 2002, **99**:15530-15535.
16. Dean EJ, Davis JC, Davis RW, Petrov DA: **Pervasive and persistent redundancy among duplicated genes in yeast.** *PLoS Genet* 2008, **4**:e1000113.
17. Musso G, Costanzo M, Huangfu M, Smith AM, Paw J, San Luis B-J, Boone C, Giaever G, Nislow C, Emili A *et al.*: **The extensive and condition-dependent nature of epistasis among whole-genome duplicates in yeast.** *Genome Res* 2008, **18**:1092-1099.
18. Deutschbauer A, Jaramillo D, Proctor M, Kumm J, Hillenmeyer M, Davis R, Nislow C, Giaever G: **Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast.** *Genetics* 2005, **169**:1915-1925.
19. Delneri D, Hoyle DC, Gkargkas K, Cross EJM, Rash B, Zeef L, Leong H-S, Davey HM, Hayes A, Kell DB *et al.*: **Identification and characterization of high-flux-control genes of yeast through competition analyses in continuous cultures.** *Nat Genet* 2008, **40**:113-117.
20. de Clare M, Oliver SG: **Copy-number variation of cancer-gene orthologs is sufficient to induce cancer-like symptoms in *Saccharomyces cerevisiae*.** *BMC Biol* 2013, **11**:24.
21. Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, Ding H, Koh JLY, Toufighi K, Mostafavi S *et al.*: **The genetic landscape of a cell.** *Science* 2010, **327**:425-431.
22. van Pel DM, Stirling PC, Minaker SW, Sipahimalani P, Hieter P: ***Saccharomyces cerevisiae* genetics predicts candidate therapeutic genetic interactions at the mammalian replication fork.** *G3* 2013, **3**:273-282.
23. McLellan JL, O'Neil NJ, Barrett I, Ferree E, van Pel DM, Ushey K, Sipahimalani P, Bryan J, Rose AM, Hieter P: **Synthetic lethality of cohesins with PARPs and replication fork mediators.** *PLoS Genet* 2012, **8**:e1002574.

6 Genetics of system biology

This study screened for synthetic lethality partners for cohesins, which have been found mutated in cancers. The interactions discovered in yeast were conserved through worms and humans, and point to using PARP inhibitors to treat tumors with cohesin deficiencies.

24. Haarer B, Viggiano S, Hibbs MA, Troyanskaya OG, Amberg DC: **Modeling complex genetic interactions in a simple eukaryotic genome: actin displays a rich spectrum of complex haploinsufficiencies.** *Genes Dev* 2007, **21**: 148-159.
 25. Haarer B, Aggeli D, Viggiano S, Burke DJ, Amberg DC: **Novel interactions between actin and the proteasome revealed by complex haploinsufficiency.** *PLoS Genet* 2011, **7**:e1002288.
 26. Stearns T, Botstein D: **Unlinked noncomplementation: isolation of new conditional-lethal mutations in each of the tubulin genes of *Saccharomyces cerevisiae*.** *Genetics* 1988, **119**: 249-260.
 27. Baetz KK, Krogan NJ, Emili A, Greenblatt J, Hieter P: **The *ctf13-30/CTF13* genomic haploinsufficiency modifier screen identifies the yeast chromatin remodeling complex RSC, which is required for the establishment of sister chromatid cohesion.** *Mol Cell Biol* 2004, **24**:1232-1244.
 28. Stankiewicz P, Lupski JR: **Structural variation in the human genome and its role in disease.** *Annu Rev Med* 2010, **61**: 437-455.
 29. Tang Y-C, Amon A: **Gene copy-number alterations: a cost-benefit analysis.** *Cell* 2013, **152**:394-405.
 30. Dymond JS, Richardson SM, Coombes CE, Babatz T, Muller H, Annaluru N, Blake WJ, Schwerzmann JW, Dai J, Lindstrom DL *et al.*: **Synthetic chromosome arms function in yeast and generate phenotypic diversity by design.** *Nature* 2011, **477**: 471-476.
 31. Gammie AE, Erdeniz N, Beaver J, Devlin B, Nanji A, Rose MD: **Functional characterization of pathogenic human *MSH2* missense mutations in *Saccharomyces cerevisiae*.** *Genetics* 2007, **177**:707-721.
 32. Ghezzi D, Baruffini E, Haack TB, Invernizzi F, Melchionda L, Dallabona C, Strom TM, Parini R, Burlina AB, Meitinger T *et al.*: **Mutations of the mitochondrial-tRNA modifier *MTO1* cause hypertrophic cardiomyopathy and lactic acidosis.** *Am J Hum Genet* 2012, **90**:1079-1087.
- This study discovered mutations in *MTO1* by exome sequencing in patients with hypertrophic cardiomyopathy. They recreated these mutations in the yeast ortholog and confirmed specific phenotypes consistent with the disease.
33. Araya CL, Fowler DM: **Deep mutational scanning: assessing protein function on a massive scale.** *Trends Biotechnol* 2011, **29**:435-442.
 34. Roscoe BP, Thayer KM, Zeldovich KB, Fushman D, Bolon DNA: **Analyses of the effects of all ubiquitin point mutants on yeast growth rate.** *J Mol Biol* 2013, **425**:1363-1377.
- This study demonstrated the application of high-throughput sequencing and selection of mutant libraries in a full-length protein in yeast. Consequently, it is an important step forward in creating sequence-function maps for interpreting how coding variation impacts protein function.
35. Marini NJ, Thomas PD, Rine J: **The use of orthologous sequences to predict the impact of amino acid substitutions on protein function.** *PLoS Genet* 2010, **6**:e1000968.
 36. Tugendreich S, Bassett DE, McKusick VA, Boguski MS, Hieter P: **Genes conserved in yeast and humans.** *Human Molecular Genetics* 1994, **3 Spec No**:1509-1517.
 37. Zeng Q, Morales AJ, Cottarel G: **Fungi and humans: closer than you think.** *Trends Genet* 2001, **17**:682-684.
 38. Zhang N, Bilisland E: **Contributions of *Saccharomyces cerevisiae* to understanding mammalian gene function and therapy.** *Methods Mol Biol* 2011, **759**:501-523.
 39. Osborn MJ, Miller JR: **Rescuing yeast mutants with human genes.** *Brief Funct Genom Proteom* 2007, **6**:104-111.
 40. Heinicke S, Livstone MS, Lu C, Oughtred R, Kang F, Angiuoli SV, White O, Botstein D, Dolinski K: **The Princeton Protein Orthology Database (P-POD): a comparative genomics analysis tool for biologists.** *PLoS ONE* 2007, **2**:e766.
 41. Zhang N, Osborn M, Gitsham P, Yen K, Miller JR, Oliver SG: **Using yeast to place human genes in functional categories.** *Gene* 2003, **303**:121-129.
 42. Tugendreich S, Perkins E, Couto J, Barthmaier P, Sun D, Tang S, Tulac S, Nguyen A, Yeh E, Mays A *et al.*: **A streamlined process to phenotypically profile heterologous cDNAs in parallel using yeast cell-based assays.** *Genome Res* 2001, **11**:1899-1912.
 43. Kruger WD, Cox DR: **A yeast system for expression of human cystathionine beta-synthase: structural and functional conservation of the human and yeast genes.** *Proc Natl Acad Sci USA* 1994, **91**:6614-6618.
 44. Kruger WD, Cox DR: **A yeast assay for functional detection of mutations in the human cystathionine β -synthase gene.** *Human Molecular Genetics* 1995, **4**:1155-1161.
 45. Mayfield JA, Davies MW, Dimster-Denk D, Pleskac N, McCarthy S, Boydston EA, Fink L, Lin XX, Narain AS, Meighan M *et al.*: **Surrogate genetics and metabolic profiling for characterization of human disease alleles.** *Genetics* 2012, **190**:1309-1323.
- This study examined the phenotype of 86 alleles of the human *CBS* gene in yeast using a cross-species complementation approach. They found a subset of alleles whose effects can be mitigated by adding vitamin B6 or heme, cofactors of the enzyme.
46. Franssens V, Bynens T, Van den Brande J, Vandermeeren K, Verduyck M, Winderickx J: **The benefits of humanized yeast models to study Parkinson's disease.** *Oxid Med Cell Longev* 2013, **2013**:760629.
 47. Clapp C, Portt L, Khoury C, Sheibani S, Eid R, Greenwood M, Vali H, Mandato CA, Greenwood MT: **Untangling the roles of anti-apoptosis in regulating programmed cell death using humanized yeast cells.** *Front Oncol* 2012, **2**:59.
 48. Lamesch P, Li N, Milstein S, Fan C, Hao T, Szabo G, Hu Z, Venkatesan K, Bethel G, Martin P *et al.*: **hORFeome v3.1: a resource of human open reading frames representing over 10,000 human genes.** *Genomics* 2007, **89**:307-315.
 49. Project Team MGC, Temple G, Gerhard DS, Rasooly R, Feingold EA, Good PJ, Robinson C, Mandich A, Derge JG, Lewis J *et al.*: **The completion of the Mammalian Gene Collection (MGC).** *Genome Res* 2009, **19**:2324-2333.
 50. Kato S, Han S-Y, Liu W, Otsuka K, Shibata H, Kanamaru R, Ishioka C: **Understanding the function-structure and function-mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis.** *Proc Natl Acad Sci USA* 2003, **100**:8424-8429.
 51. Baronio R, Danziger SA, Hall LV, Salmon K, Hatfield GW, Lathrop RH, Kaiser P: **All-codon scanning identifies p53 cancer rescue mutations.** *Nucleic Acids Res* 2010, **38**: 7079-7088.
 52. Otsuka K, Kato S, Kakudo Y, Mashiko S, Shibata H, Ishioka C: **The screening of the second-site suppressor mutations of the common p53 mutants.** *Int J Cancer* 2007, **121**:559-566.
 53. Kakudo Y, Shibata H, Otsuka K, Kato S, Ishioka C: **Lack of correlation between p53-dependent transcriptional activity and the ability to induce apoptosis among 179 mutant p53s.** *Cancer Res* 2005, **65**:2108-2114.
 54. Couthouis J, Hart MP, Shorter J, DeJesus-Hernandez M, Erion R, Oristano R, Liu AX, Ramos D, Jethava N, Hosangadi D *et al.*: **A yeast functional screen predicts new candidate ALS disease genes.** *Proc Nat Acad Sci USA* 2011, **108**:20881-20890.
- This paper used a heterologous expression assay in yeast to screen 133 candidate proteins with similar features to known ALS genes. One of the candidates discovered to aggregate and cause toxicity in yeast was found to be mutated in ALS patients whose disease had an unknown cause.
55. Tarassov K, Messier V, Landry CR, Radinovic S, Serna Molina MM, Shames I, Malitskaya Y, Vogel J, Bussey H, Michnick SW: **An in vivo map of the yeast protein interactome.** *Science* 2008, **320**:1465-1470.

56. Fields S, Song O: **A novel genetic system to detect protein-protein interactions.** *Nature* 1989, **340**:245-246.
57. Gislser SM, Kittanakom S, Fuster D, Wong V, Bertic M, Radanovic T, Hall RA, Murer H, Biber J, Markovich D *et al.*: **Monitoring protein-protein interactions between the mammalian integral membrane transporters and PDZ-interacting partners using a modified split-ubiquitin membrane yeast two-hybrid system.** *Mol Cell Proteomics* 2008, **7**:1362-1377.
58. Lenk GM, Ferguson CJ, Chow CY, Jin N, Jones JM, Grant AE, Zolov SN, Winters JJ, Giger RJ, Dowling JJ *et al.*: **Pathogenic mechanism of the FIG4 mutation responsible for Charcot-Marie-Tooth disease CMT4J.** *PLoS Genet* 2011, **7**:e1002104.
59. Woods JO, Singh-Blom UM, Laurent JM, McGary KL,
 • Marcotte EM: **Prediction of gene-phenotype associations in humans, mice, and plants using phenologs.** *BMC Bioinformatics* 2013, **14**:203.
- This paper continued to develop the phenolog concept first laid out in this group's 2010 PNAS paper [60]. The improved method can take into account more data types, including 'paralogous' phenotypes.
60. McGary KL, Park TJ, Woods JO, Cha HJ, Wallingford JB, Marcotte EM: **Systematic discovery of nonobvious human disease models through orthologous phenotypes.** *Proc Natl Acad Sci USA* 2010, **107**:6544-6549.
61. Lehner B: **Genotype to phenotype: lessons from model organisms for human genetics.** *Nat Rev Genet* 2013, **14**:168-178.
62. Zuk O, Hechter E, Sunyaev SR, Lander ES: **The mystery of missing heritability: genetic interactions create phantom heritability.** *Proc Natl Acad Sci USA* 2012, **109**:1193-1198.
63. Kvittek DJ, Sherlock G: **Reciprocal sign epistasis between frequently experimentally evolved adaptive mutations causes a rugged fitness landscape.** *PLoS Genet* 2011, **7**:e1002056.
64. Bloom JS, Ehrenreich IM, Loo WT, Lite T-LV, Kruglyak L: **Finding the sources of missing heritability in a yeast cross.** *Nature* 2013, **494**:234-237.
- This study crossed two divergent strains of yeast and measured the growth phenotypes of over 1000 segregants in 46 conditions. They found that heritability varied depending on the trait, but could largely be accounted for. The degree of the heritability explained by simple additive effects versus gene-gene interactions also varied considerably.
65. Dowell RD, Ryan O, Jansen A, Cheung D, Agarwala S, Danford T, Bernstein DA, Rolfe PA, Heisler LE, Chin B *et al.*: **Genotype to phenotype: a complex problem.** *Science* 2010, **328**:469.
66. Skelly DA, Merrihew GE, Riffle M, Connelly CF, Kerr EO, Johansson M, Jaschob D, Graczyk B, Shulman NJ, Wakefield J *et al.*: **Integrative phenomics reveals insight into the structure of phenotypic diversity in budding yeast.** *Genome Res* 2013, **23**:1496-1504.