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Contemporary, yeast-based approaches to understanding human genetic variation

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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.

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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

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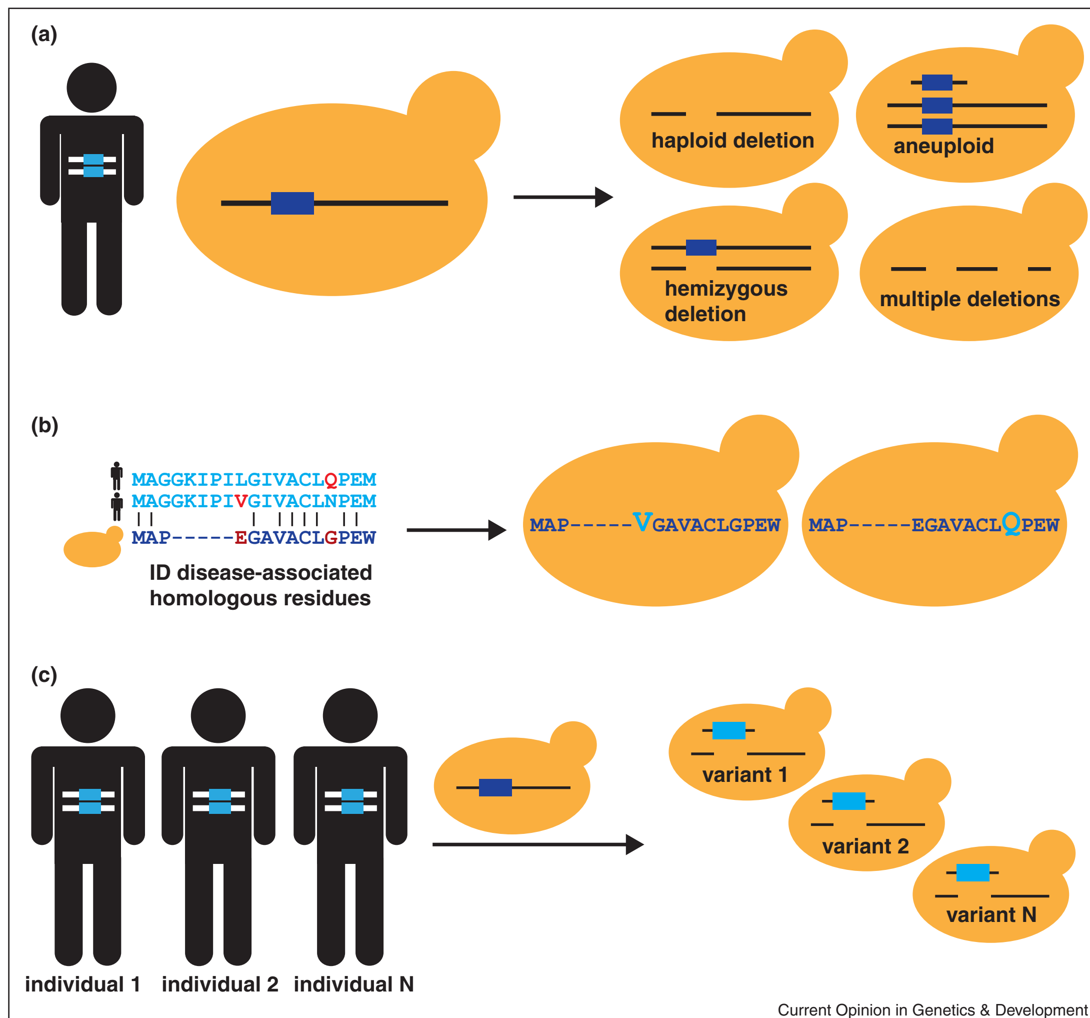
Figure 1



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 knock-out 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

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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

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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 in 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

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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.

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