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From drug to protein: using yeast genetics for high-throughput target discovery

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The budding yeast *Saccharomyces cerevisiae* has long been an effective eukaryotic model system for understanding basic cellular processes. The genetic tractability and ease of manipulation in the laboratory make yeast well suited for large-scale chemical and genetic screens. Several recent studies describing the use of yeast genetics for high-throughput drug target identification are discussed in this review.

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Introduction

The inhibitory and stimulatory effects of organic small molecules are fundamental to the therapeutic intervention of disease states. Although effective therapeutic agents can be developed and successfully administered without knowledge of the underlying mechanism of action, elucidation of drug target proteins can lead to the design of new compounds with improved safety and efficacy profiles [1]. However, identification of small-molecule targets remains one of the most difficult and challenging tasks that researchers face. Traditional biochemical approaches for target identification rely mainly on *in vitro* binding assays that are both time consuming and labor intensive. Powerful new approaches that combine model organism genetics with new techniques in molecular biology and computational genomics are potent alternatives to these older technologies as a first step in the target discovery process.

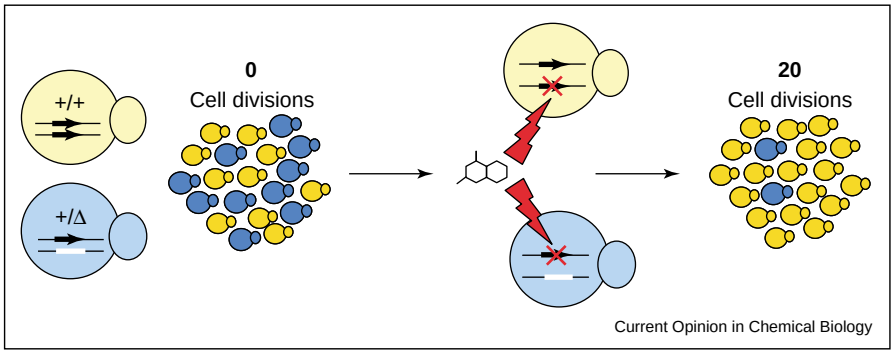
The yeast *Saccharomyces cerevisiae* has been used successfully for many years as a model organism for mammalian diseases and pathways [2]. In fact, the cellular target of rapamycin, an immunosuppressant used as an anti-rejection drug in tissue transplants, was first discovered in yeast and subsequently verified in humans [3,4]. The availability of large, well-characterized mutant strain collections and gene libraries make yeast particularly well suited to large-scale chemical-genetic screens. Completion of the yeast genome sequence in 1996 led to an effort to systematically disrupt each gene in the genome by gene replacement [5]. The resulting strain collection consists of isogenic homozygous and heterozygous mutants, each being deficient in one of the nearly 6000 genes encoded in the yeast genome. A unique DNA sequence tag embedded in the genome of each strain can be used to quickly genotype any mutant strain by PCR. The application of innovative technologies such as DNA microarrays provides new avenues for genetic screens using these mutant strain collections [6–9]. In this review, we focus on three highly scalable approaches using yeast genetics for drug target discovery.

Drug-induced haploinsufficiency

The use of gene dosage effects to isolate drug targets in yeast was first documented over 20 years ago [10]. In that pivotal study, the protein targets of two compounds, compactin and tunicamycin, were identified by screening a genomic library for genes that confer drug-resistance when overexpressed. Yeast clones harboring multiple copies of a target gene were less sensitive to the compound than those expressing non-target genes. In principle, this approach can be applied to any compound targeting a yeast protein, but the broad application of this method is partially limited by the fact that some genes are toxic when overexpressed. This necessitated the development of a complementary method that would allow identification of genes that sensitize a yeast cell to a drug by reducing target gene dosage. In contrast to overexpression studies where the desired phenotype is selectable, drug sensitivity screens present a unique challenge to classical genetic strategies. With standard yeast genetic screens, a mutagenized yeast strain is transferred from rich to drug-containing media by replica plating. Hypersensitive colonies are then transformed with a genomic library to identify clones that rescue the phenotype by functional complementation of the mutated gene. This process requires a series of subsequent validation steps and can yield a large number of false positive genes that do not encode the actual drug target.

Strain collections consisting of single gene deletion mutants for all of the nearly 6000 genes in the yeast genome now make it possible to quickly assess drug-sensitivities on a large scale [11••,12•,13]. The use of heterozygotes has proven to be particularly powerful for drug target-identification *in vivo*. In a landmark study,

Figure 1



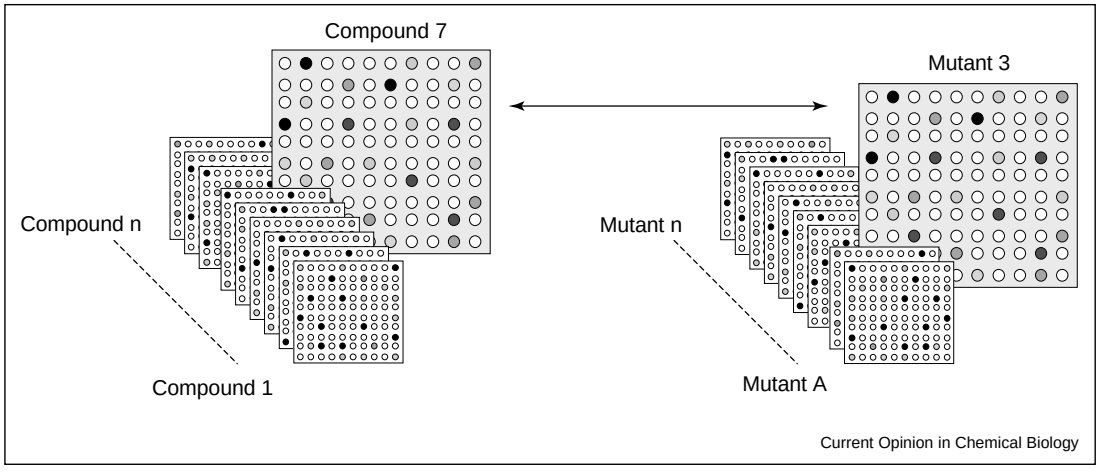
Drug-induced haploinsufficiency. Reduction of target gene copy number can sensitize diploid cells to a drug. Competitive growth experiments can exploit the drug-dependent growth differences between heterozygotes and wild-type cells.

Giaever *et al.* demonstrated that simply reducing the copy number of a drug target gene from two to one can significantly sensitize a diploid cell to drug [14]. This phenomenon, known as drug-induced haploinsufficiency, was used to identify the known targets of a few well-characterized compounds using competitive growth experiments with small pools of heterozygous deletion mutants (Figure 1). Strain-specific molecular tags hybridized to DNA microarrays were used to monitor the growth of individual heterozygotes before and after drug treatment. Recently, this approach has been extended to larger strain pools and diverse compound libraries to identify novel drug targets. In a study by Lum *et al.*, a pool of nearly 4000 heterozygotes was tested against 78 diverse chemical treatments [11••]. Statistical analysis of the resulting microarray data revealed a small number of highly specific drug-sensitive strains for about 50 of the compounds tested. Targets were correctly identified for most of the compounds with known protein interactions. Novel candidate targets of two therapeutic compounds, the anti-anginal compound molsidomine and the anti-proliferative agent 5-fluorouracil, were uncovered and later confirmed with conventional molecular biology and biochemical methods. In a parallel study by Giaever *et al.*, a smaller set of 10 compounds was analyzed against a pool consisting of heterozygotes representing nearly every gene in the yeast genome [12•]. Most of the compounds that were profiled in both of these studies yielded similar target candidates, demonstrating the robustness and scalability of this assay. Slightly different applications of the approach using laboratory automation and robotics can also be used for large-scale screens of drug-induced haploinsufficiency [13].

Pattern matching

Another useful development in target discovery has been the marriage of large-scale experiments and computational analysis to link complex biological patterns generated by chemical and genetic perturbations (Figure 2). For instance, Hughes *et al.* described the use of a large

Figure 2



Complex phenotype pattern matching. Drug-sensitivity profiles of gene deletion strains can be matched to synthetic lethal patterns of drug target genes by clustering analysis. Profiles can be generated from a variety of cellular readouts including gene expression or strain fitness.

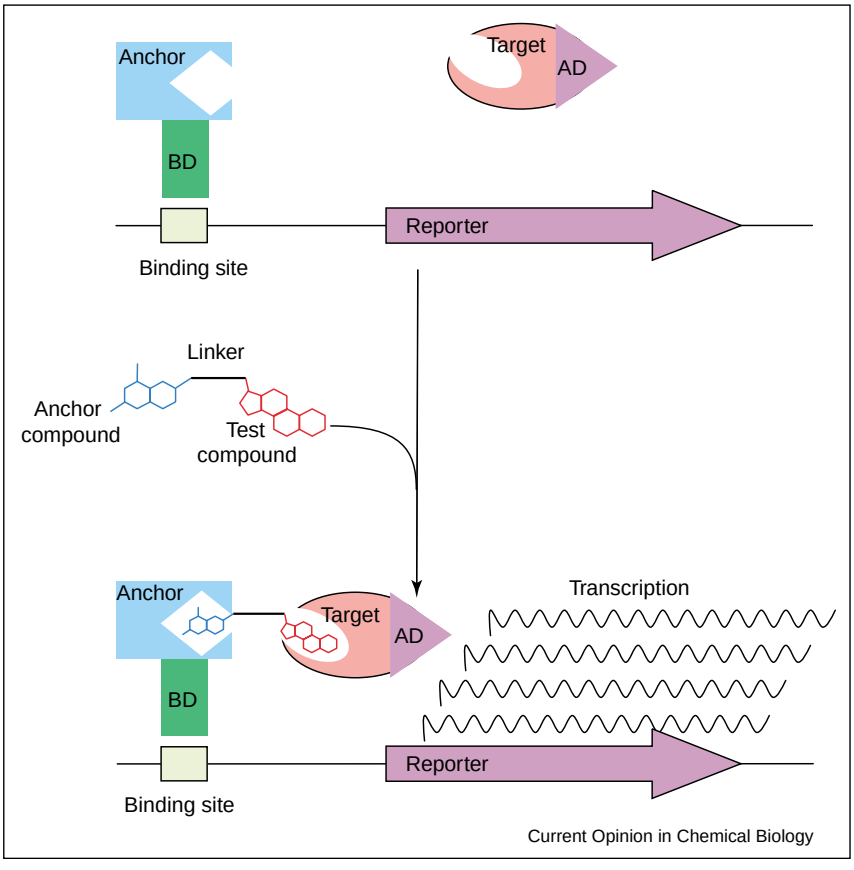
compendium of gene expression profiles to identify genes and pathways affected by bioactive compounds [15]. This approach used two-dimensional hierarchical clustering of a diverse collection of 300 full-genome expression profiles to match drug treatments with yeast mutants deficient in a target protein.

More recently, the concept of complex pattern matching was expanded to comparing patterns of genetic- and compound-induced synthetic lethality. In a compelling proof-of-principle study, ordered arrays of ~4700 yeast deletion mutants [16] were used to generate synthetic lethal profiles for several known drug target genes [17••]. The expression levels of essential genes were lowered conditionally via a titratable promoter [18]. Ten chemical treatments were done in parallel with the same set of mutant strains to generate drug-sensitivity profiles. Clustering of the synthetic lethal profiles with the chemical profiles resulted in the identification of the known target (*ERG11*) of the antifungal drug fluconazole. In addition, clustering analysis revealed a set of multi-drug resistance genes that were filtered out in subsequent analyses. When expanded to the full-genome level, synthetic lethal profiles of every gene in the genome could theoretically be used to query compounds with unknown mechanisms of action. In addition to target discovery, complex phenotypes such as gene expression signatures and synthetic lethal profiles can be exploited to understand gene function by network analysis [17••,19•,20].

Small-molecule three-hybrid

In a more direct approach to mammalian target discovery, the yeast three-hybrid approach exploits molecular and genetic tools developed for the detection of protein–protein interactions with the yeast two-hybrid system [21]. This method can be used to directly query the mammalian proteome for small-molecule targets. With the three-hybrid system, a hybrid compound consisting of two small molecules is used to reconstitute a functional transcription factor that drives expression of a reporter gene (Figure 3). Dimerization of the transcriptional components occurs through two independent small-molecule protein interactions. A known ligand–receptor interaction such as dexamethasone-glucocorticoid receptor can be used as an anchor from which a test compound can be screened for interacting proteins. Binding of a target

Figure 3



Small-molecule three-hybrid for the detection of compound–protein interactions. Activation of a reporter gene is dependent on an interaction between a test compound and an unknown target protein. AD, transcriptional activation domain; BD, DNA binding domain.

protein to the test compound results in expression of the reporter construct.

Several proof-of-principle experiments have demonstrated the potential utility of small molecule three-hybrid systems [22•,23–25] and recent work has employed one version of this system to successfully screen cDNA libraries for novel compound–protein interactions [26••]. Becker *et al*. used methotrexate as an anchor to identify known and novel targets of cyclin-dependent kinase inhibitors from several human cDNA libraries. In this approach, the LexA DNA binding domain and the GAL4 activation domain formed the basis of a positive selection scheme using the selectable auxotrophic marker HIS3 as a reporter. Several candidate targets resulting from the screen were independently confirmed using conventional biochemical methods. Variations using different combinations of reporter genes, anchor interactions and transcriptional domains provide an array of options available to the experimental design of three-hybrid screens. An abundance of cDNA libraries constructed specifically for use with the two-hybrid system are widely available for use here. Careful selection and construction of system components such as anchor compound–protein interactions and yeast strain background are important determinants of assay sensitivity and specificity. Although it is unclear how broadly this method can be applied to chemically and structurally diverse compounds, the three-hybrid strategy promises to be a valuable tool in future drug-development efforts.

Conclusions

Several high-throughput *in vivo* screening approaches have been developed for the identification of drug targets in yeast. These approaches enable researchers to identify protein targets and pathways of bioactive compounds without *a priori* knowledge of the underlying mechanisms of action. For example, yeast proteins targeted by compounds can be identified by methods such as drug-induced haploinsufficiency and complex phenotype pattern matching. These strategies have been validated through successful identification of known and novel drug targets. However, these techniques do have shortcomings that are inherent to yeast genetics. For example, some drug targets may not be encoded in the relatively small yeast genome and target gene phenotypes may be masked by genetic and functional redundancy. In addition, results may not translate to more complex eukaryotes, hence additional validation steps are required. Alternative approaches such as three-hybrid analysis do allow direct identification of mammalian targets through positive selection strategies. Small molecule three-hybrid circumvents the need for cross-species validation of targets, but it depends on custom syntheses of chemical probes. Ectopic expression of proteins can also be problematic in some cases. Although we have highlighted some obvious drawbacks, the unbiased nature of these *in vivo* detection systems are most powerful when applied in combination with complementary genomic and proteomic approaches in a systems biology setting [27,28]. Hypotheses generated by modern applications in yeast genetics will no doubt contribute valuable insights to the spectrum of cellular activities induced by small-molecule therapeutics.

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References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Tunaru S, Kero J, Schaub A, Wufka C, Blaukat A, Pfeffer K, Offermanns S: **PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect**. *Nat Med* 2003, **9**:352-355.

2. Hartwell LH: **Nobel Lecture. Yeast and cancer**. *Biosci Rep* 2002, **22**:373-394.

3. Heitman J, Movva NR, Hall MN: **Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast**. *Science* 1991, **253**:905-909.

4. Schreiber SL, Crabtree GR: **The mechanism of action of cyclosporin A and FK506**. *Immunol Today* 1992, **13**:136-142.

5. Winzeler 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.

6. Butcher RA, Schreiber SL: **Identification of Ald6p as the target of a class of small-molecule suppressors of FK506 and their use in network dissection**. *Proc Natl Acad Sci USA* 2004, **101**:7868-7873.

7. Zewail A, Xie MW, Xing Y, Lin L, Zhang PF, Zou W, Saxe JP, Huang J: **Novel functions of the phosphatidylinositol metabolic pathway discovered by a chemical genomics screen with wortmannin**. *Proc Natl Acad Sci USA* 2003, **100**:3345-3350.

8. Wright R, Parrish ML, Cadera E, Larson L, Matson CK, Garrett-Engele P, Armour C, Lum PY, Shoemaker DD: **Parallel analysis of tagged deletion mutants efficiently identifies genes involved in endoplasmic reticulum biogenesis**. *Yeast* 2003, **20**:881-892.

9. Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B *et al.*: **Functional profiling of the *Saccharomyces cerevisiae* genome**. *Nature* 2002, **418**:387-391.

10. Rine J, Hansen W, Hardeman E, Davis RW: **Targeted selection of recombinant clones through gene dosage effects**. *Proc Natl Acad Sci USA* 1983, **80**:6750-6754.

11. •• Lum PY, Armour CD, Stepaniants SB, Cavet G, Wolf MK, Butler JS, Hinshaw JC, Garnier P, Prestwich GD, Leonardson A *et al.*: **Discovering modes of action for therapeutic compounds using a genome-wide screen of yeast heterozygotes**. *Cell* 2004, **116**:121-137.
Using a highly parallel approach with barcoded yeast heterozygote strains, microarrays, and 78 compounds, these authors used haploinsufficiency to identify novel targets for known compounds.

12. • Giaever G, Flaherty P, Kumm J, Proctor M, Nislow C, Jaramillo DF, Chu AM, Jordan MI, Arkin AP, Davis RW: **Chemogenomic profiling: identifying the functional interactions of small molecules in yeast**. *Proc Natl Acad Sci USA* 2004, **101**:793-798.
Approach similar to the [11••] but with 10 compounds and nearly every deletion strain in the yeast genome.

13. Baetz K, McHardy L, Gable K, Tarling T, Reberioux D, Bryan J, Andersen RJ, Dunn T, Hieter P, Roberge M: **Yeast genome-wide drug-induced haploinsufficiency screen to determine drug mode of action**. *Proc Natl Acad Sci USA* 2004, **101**:4525-4530.

14. Giaever G, Shoemaker DD, Jones TW, Liang H, Winzeler EA, Astromoff A, Davis RW: **Genomic profiling of drug sensitivities via induced haploinsufficiency**. *Nat Genet* 1999, **21**:278-283.

15. Hughes TR, Marton MJ, Jones AR, Roberts CJ, Stoughton R, Armour CD, Bennett HA, Coffey E, Dai H, He YD *et al.*: **Functional discovery via a compendium of expression profiles**. *Cell* 2000, **102**:109-126.

16. Tong AH, Evangelista M, Parsons AB, Xu H, Bader GD, Page N, Robinson M, Raghibizadeh S, Hogue CW, Bussey H *et al.*: **Systematic genetic analysis with ordered arrays of yeast deletion mutants**. *Science* 2001, **294**:2364-2368.

17. •• Parsons AB, Brost RL, Ding H, Li Z, Zhang C, Sheikh B, Brown GW, Kane PM, Hughes TR, Boone C: **Integration of chemical-genetic and genetic interaction data links bioactive compounds to cellular target pathways**. *Nat Biotechnol* 2004, **22**:62-69.
Comparison of drug sensitivity and synthetic lethal profiles to identify drug targets and relevant pathways.

18. Mnaimneh S, Davierwala AP, Haynes J, Moffat J, Peng WT, 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.

19. • Haggarty SJ, Clemons PA, Schreiber SL: **Chemical genomic profiling of biological networks using graph theory and combinations of small molecule perturbations**. *J Am Chem Soc* 2003, **125**:10543-10545.
Using drug sensitivity profiles of mutant yeast strains to study gene interaction networks.

20. Ooi SL, Shoemaker DD, Boeke JD: **DNA helicase gene interaction network defined using synthetic lethality analyzed by microarray**. *Nat Genet* 2003, **35**:277-286.

21. Fields S, Song O: **A novel genetic system to detect protein-protein interactions**. *Nature* 1989, **340**:245-246.

22. • Henthorn DC, Jaxa-Chamiec AA, Meldrum E: **A GAL4-based yeast three-hybrid system for the identification of small molecule-target protein interactions**. *Biochem Pharmacol* 2002, **63**:1619-1628.
Used a three-hybrid approach to identify the target of methotrexate from a mammalian cDNA library.

23. Baker K, Sengupta D, Salazar-Jimenez G, Cornish VW: **An optimized dexamethasone-methotrexate yeast 3-hybrid system for high-throughput screening of small molecule-protein interactions**. *Anal Biochem* 2003, **315**:134-137.

24. Abida WM, Carter BT, Althoff EA, Lin H, Cornish VW: **Receptor-dependence of the transcription read-out in a small-molecule three-hybrid system**. *ChemBioChem* 2002, **3**:887-895.

25. Licitra EJ, Liu JO: **A three-hybrid system for detecting small ligand-protein receptor interactions**. *Proc Natl Acad Sci USA* 1996, **93**:12817-12821.

26. •• Becker F, Murthi K, Smith C, Come J, Costa-Roldan N, Kaufmann C, Hanke U, Degenhart C, Baumann S, Wallner W *et al.*: **A three-hybrid approach to scanning the proteome for targets of small molecule kinase inhibitors**. *Chem Biol* 2004, **11**:211-223.
Mammalian cDNA library screen identified known and novel targets of CDK inhibitors.

27. Auffray C, Imbeaud S, Roux-Rouquie M, Hood L: **From functional genomics to systems biology: concepts and practices**. *C R Biol* 2003, **326**:879-892.

28. Verhoeckx KC, Bijlsma S, Jespersen S, Ramaker R, Verheij ER, Witkamp RF, Van Der Greef J, Rodenburg RJ: **Characterization of anti-inflammatory compounds using transcriptomics, proteomics, and metabolomics in combination with multivariate data analysis**. *Int Immunopharmacol* 2004, **4**:1499-1514.