

Minireview

## Chemical genomics in yeast

Charles Brenner

Address: Departments of Genetics and Biochemistry and the Norris Cotton Cancer Center, Dartmouth Medical School, Lebanon, NH 03756, USA. E-mail: charles.brenner@dartmouth.edu

Published: 27 August 2004

*Genome Biology* 2004, **5**:240

The electronic version of this article is the complete one and can be found online at <http://genomebiology.com/2004/5/9/240>

© 2004 BioMed Central Ltd

### Abstract

Many drugs have unknown, controversial or multiple mechanisms of action. Four recent 'chemical genomic' studies, using genome-scale collections of yeast gene deletions that were either arrayed or barcoded, have presented complementary approaches to identifying gene-drug and pathway-drug interactions.

The fact that much of modern drug research is target-oriented obscures the long history during which the effects of drugs were discovered prior to identification of their targets. There remain many natural products and drugs for which the cellular target protein(s) are yet to be fully characterized. Without the identification of specific targets, it is extremely difficult to modify and improve the performance of drugs and to determine whether side effects are due to effects on the primary target or 'off target' effects. Indeed, mouse-on-the-hotplate analgesia assays [1] seem crude in this era of high-throughput screening and structure-based refinement of, for example, inhibitors of cyclooxygenase 2 (COX-2) [2]. But the advantage of targetless whole-animal assays is that compounds that would pass some early stage of *in vitro* screening, only later to fail to have *in vivo* efficacy, do not score positively in whole-animal assays. This is important because acceleration of failure is considered to be a cost- and time-conserving necessity in drug discovery.

There is clearly a need to work through the legacy compound collections owned by pharmaceutical companies. In addition, the introduction of zebrafish [3] and invertebrate [4] systems into drug screening (for the advantages cited above for mouse assays) will ensure that there is an ongoing need for genetic and genomic strategies that can identify the targets of pharmacologically interesting small molecules. Because no eukaryotic model system is more genetically advanced than the budding yeast *Saccharomyces cerevisiae* and because fungi contain substantial numbers of orthologs of human drug targets, baker's yeast is an obviously place to

start to identify drug mechanisms of action. Four recent papers [5-8] make important contributions to mapping drug effects using yeast genomics.

### The genetic basis of drug sensitivity and resistance

Before reviewing recent work, it is useful to consider a number of ways in which a drug might inhibit the division, growth or survival of yeast cells. Conceptually, the simplest mechanism is that a drug inhibits an enzyme essential for cell division and growth, such as a replicative DNA polymerase. If this were the case, then the primary target of the drug is expected to be encoded by an essential gene, such that one could select for dominant resistant mutants in haploid or diploid cells; but one could not easily conduct a screen that would depend on recovery of haploid loss-of-function mutants in the target gene because such cells are expected to be nonviable. Because a collection of all potential dominant mutants would have many more members than the total number of genes (and, in fact, many more than the total number of codons), there is no standing genetic resource for such drug selections in any organism. Typically, a researcher interested in selecting for dominant drug-resistant mutants will mutagenize cells anew and plate many millions of such cells for resistant colonies.

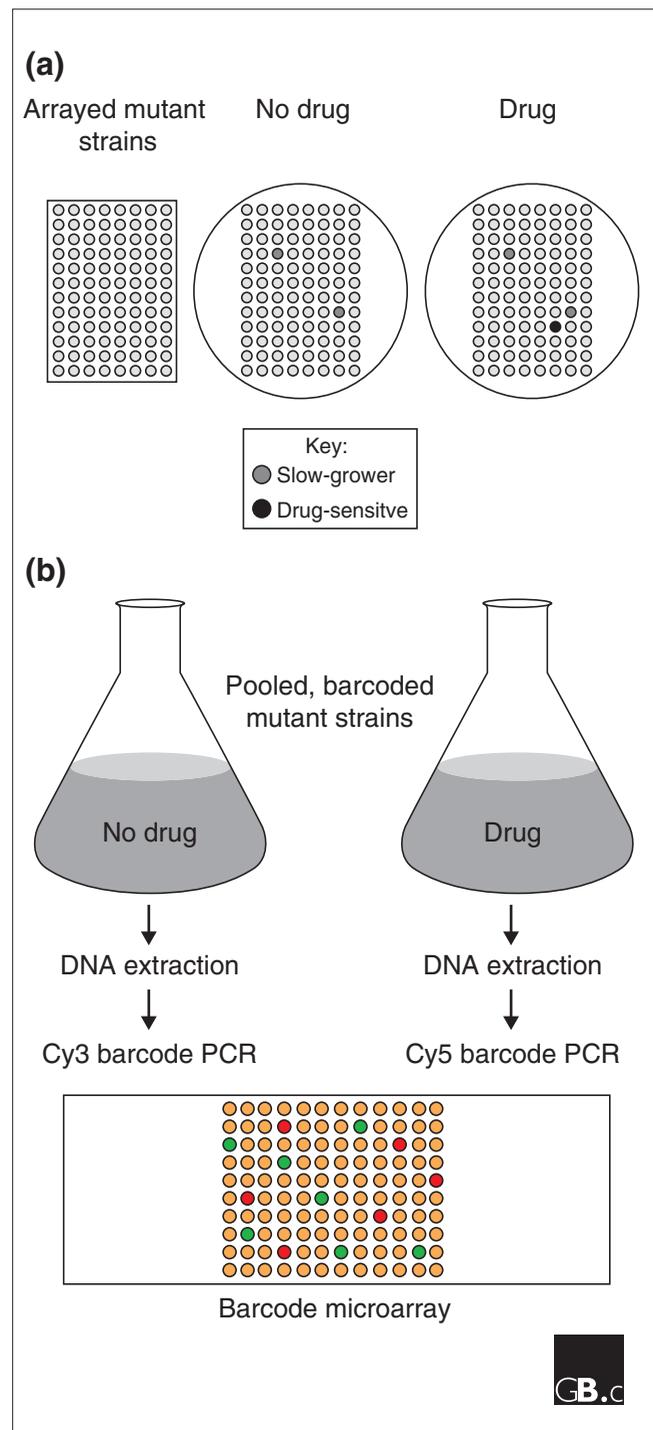
In the case of replicative polymerases, many drugs that act on these proteins are actually pro-drugs, compounds that depend on cellular enzymes for conversion to the active

inhibitory agents. Thus, for azidothymidine or dideoxyinosine to inhibit their intended targets (reverse transcriptases) or their unintended targets (host DNA polymerases), the compounds must undergo three cycles of phosphorylation to generate chain-terminating nucleoside triphosphates. For cases in which pro-drug activation is limiting for toxicity, simple loss-of-function mutations in pro-drug activation enzymes are expected to cause drug resistance, unless the enzymes are encoded by essential genes, while supersensitive mutants might be identified as specific gain-of-function alleles of pro-drug activating enzymes. The ability to select a resistant mutant as a simple loss-of-function allele is a tremendous advantage because the necessary mutagenized library size becomes equal to the number of nonessential genes.

Identifying drug-sensitive mutants from libraries of loss-of-function mutants has been facilitated by the preparation of nearly complete collections of yeast gene knockouts. For drug screens in which one wishes to query only nonessential genes, haploid yeast libraries can be used that will ultimately number around 5,000 strains, each carrying a deletion of a single nonessential gene. Typically, the gene-drug interactions that are discovered from such screens do not point directly to the target of the drug being screened but rather to a second cellular process that is rendered essential by treatment with a drug. By contrast, for drug screens in which one would like to embrace the full complement of nonessential and essential genes, libraries of yeast heterozygous diploid strains can be utilized that will ultimately number more than 6,000. Screening such heterozygous libraries typically identifies the drug target by a process termed drug-induced haploinsufficiency [9], but titration of a target by a drug may also render another gene or pathway 'synthetically' lethal.

### Genetic arraying and barcoding

Historically, experiments that positively select for features of interest have been considered to be more powerful than simple mutant screens because more cells can be plated in a selection (for colony formation against a background of drug sensitivity) than in a screen (failure to grow against a background of growth). The problem is recovering and identifying the most sensitive strains from screens. There are two practical approaches to this problem: genetic arraying [5,6] and barcoding [7,8], which are schematized in Figure 1. In a genetic arraying experiment, thousands of strains of different genotypes are maintained in ordered grids and transferred to drug plates, typically by robotic transfer. This approach was first utilized in genome-scale two-hybrid [10] and synthetic-lethal analyses [11]. While the genetic array may be at a genomic scale, I prefer the term 'genetic array' to 'genomic array', to avoid confusion with expression arrays and to emphasize that the arrayed elements are mutant strains. Alternatively, in a barcoding experiment, a genome-scale deletion library is treated as a single pool. Because, in the construction of the collection of yeast gene deletions,



**Figure 1**  
Genetic arrays and barcodes for chemical genomics. **(a)** In a genetic array, constructed mutants are maintained separately and are stamped onto no-drug or drug-containing petri dishes for time-dependent photographic analysis. **(b)** In a barcoding experiment, mutants are pooled and grown together with and without drugs. Growth of each of the mutants is assessed by hybridization of amplified barcodes to a microarray (the barcodes are labeled with Cy3 or Cy5 fluorescent dyes). Because particular mutant strains may have drug-free growth defects and/or lower barcode microarray signal efficiency, drug sensitivity or resistance can only be scored for a particular strain in comparison to its behavior without a drug.

oligonucleotide 'barcodes' were integrated next to each deletion and flanked by common PCR-primer annealing sites [12,13], loss of a strain heterozygous for a particular gene can be measured by loss of signal in a DNA microarray using a barcode oligonucleotide chip [9]. The barcode microarray is not an expression array but rather a microarray performed to quantify introduced DNA elements in the mixed population of strains. Both genetic arrays and barcoding have recently been used to identify drug targets using yeast.

### Sensitivity of tryptophan auxotrophs to ibuprofen

Using a genetic-array-based method, Tucker and Fields [5] investigated the sensitivity of 4,800 haploid yeast strains, each containing a deletion of a different gene, to ibuprofen and three other drugs. After removing strains considered too sick for scoring drug effects and those that were sensitive to multiple drugs, the authors found a number of interesting gene-drug and pathway-drug interactions. Ibuprofen is widely used as a nonsteroidal anti-inflammatory drug (NSAID) that inhibits cyclooxygenase. The NSAIDs ibuprofen, indomethacin and sulindac sulfide, however, have been reported to reduce Alzheimer's disease and amyloidogenesis in a manner that does not involve cyclooxygenase [14], and it appears that the cancer chemopreventive effects of NSAIDs are not entirely due to cyclooxygenase inhibition [15]. As yeast cells do not encode a cyclooxygenase but 50  $\mu$ M ibuprofen is growth-inhibitory to wild-type strains, it was interesting to examine whether mutations in any nonessential yeast gene(s) conferred increased sensitivity to ibuprofen.

Remarkably, mutations in any of seven different genes involved in tryptophan biosynthesis conferred enhanced sensitivity to ibuprofen [5]. It is important to note that this does not indicate that ibuprofen inhibits tryptophan biosynthesis - all of these strains are viable on rich media - but that loss of tryptophan synthesis coupled with the inhibitory effects of ibuprofen on another molecule is toxic. In fact, given the way this experiment was done, the existence of a deletion strain that is synthetically lethal or synthetically less viable with this set of strains could potentially identify the direct target of ibuprofen toxicity in yeast. Alternatively, overexpression-cloning could potentially identify the same molecule as an ibuprofen-resistant transformant.

### Targeting of sphingolipid synthesis by dihydromotuporamine C

Using a genetic arraying method, Baetz and co-workers [6], screened 5,000 heterozygous yeast mutants for sensitivity to dihydromotuporamine C, a compound used in preclinical development as an inhibitor of metastasis. These investigators did not exclude sick strains and identified 21 heterozygous mutants that exhibited the greatest drug sensitivity. Transferring the 21 identified strains to liquid cultures with and without drugs, they established that mutants heterozygous for two steps in sphingolipid metabolism were the most sensitive to dihydromotuporamine C. Turning to a candidate-gene

approach, they then discovered four additional strains heterozygous for genes involved in sphingolipid metabolism that were drug-sensitive. Because, additionally, the drug reduced ceramide production in wild-type yeast and addition of 50 nM ceramide protected human breast carcinoma cell line MDA-231 from the drug, it appears that dihydromotuporamine C targets sphingolipid biosynthesis directly.

### Rediscovery of rRNA processing as a target of 5-fluorouracil

Using the barcoding method and a collection of 3,500 heterozygous yeast diploid strains, Lum and colleagues [7] screened 78 different drugs, the majority of which are approved by the US Food and Drug Administration (FDA) and are considered to have well-characterized targets. Among these drugs was the cancer chemotherapy agent 5-fluorouracil, which was shown more than 30 years ago to inhibit rRNA processing [16]. Despite extensive studies of the drug's mechanism of action in fungal [17], fly [18] and human [16] systems, this information was nearly, but not entirely, forgotten [19] as investigators focused on the potential for 5-fluorouracil to function as a pro-drug of 5-fluorodUMP, a potent inhibitor of thymidylate synthetase [20]. When Lum and colleagues tested heterozygous yeast strains for sensitivity to 5-fluorouracil, they found eight strains that were more sensitive than thymidylate synthetase heterozygotes; at least seven of these mutants are defective in rRNA processing, a result that not only reinvigorates studies of the mechanism of action of 5-fluorouracil but also suggests a number of new potential cancer targets.

### A new type of structure-activity relationship analysis

Quantitative structure-activity relationship (QSAR) analysis is performed when a series of defined alterations in a small molecule are correlated with the read-out of an *in vitro* or *in vivo* assay of drug efficacy. When Giaever and co-workers [8] screened 10 different drugs against 5,900 heterozygous yeast strains by the barcoding method, they were surprised to find that dyclonine, fenpropimorph, and alverine citrate, which are described as an anesthetic, an agricultural antifungal, and a muscle relaxant, respectively, have nearly identical profiles of haploinsufficiency, with *erg24*, *set6* and *tpi18* heterozygotes being most sensitive. Furthermore, these investigators realized that the three drugs share a core substructure that may provide the genetic basis for their action. The haploinsufficiency profile can be considered an exquisitely sensitive qualitative (rather than quantitative) structure-activity relationship that maps chemical properties not onto pharmacological potencies but onto pathways. The degree to which the shared pathways in yeast underlie target or off-target effects in the use of these drugs can now be investigated.

In conclusion, although many high-throughput chemical screening methods were developed for enzyme-target-based assays, the parallelization of genetic and genomic methods have

now made possible quantification of gene-drug interactions on previously unanticipated scales. As with other areas of genomics, the major challenge of chemical genomics is not to extend the number of experiments to the factorial limit of compounds, concentrations and strains, but rather to do the most penetrating validation and mechanistic experiments to extract the greatest insight from primary screens. Both genetic-array-based and barcode-based methods can suffer from systematic artifacts in which a particular deletion strain might have been misidentified or might contain unintended additional genetic changes that are or are not linked to the intended deletion. Thus, as more experiments are performed with existing yeast genomic libraries, accessible databases should be archived with the detailed findings of previous screens. Finally, standing on the shoulders of the yeast community, investigators developing mutant resources in *Escherichia coli* [21], *Neurospora crassa* [22] and vertebrate cells are advancing genetic array [23] and barcode [24,25] methods for functional screens that will allow chemistry to interact with genomics across more branches of the tree of life.

## References

- Brands B, Hirst M, Gowdey CW, Baskerville JC: **Analgesia duration and physical dependence in mice after a single injection of three heroin salts and morphine sulphate in various vehicles.** *Arch Int Pharmacodyn Ther* 1978, **231**:285-296.
- Kalgutkar AS, Zhao Z: **Discovery and design of selective cyclooxygenase-2 inhibitors as non-ulcerogenic, anti-inflammatory drugs with potential utility as anti-cancer agents.** *Curr Drug Targets* 2001, **2**:79-106.
- Peterson RT, Shaw SY, Peterson TA, Milan DJ, Zhong TP, Schreiber SL, MacRae CA, Fishman MC: **Chemical suppression of a genetic mutation in a zebrafish model of aortic coarctation.** *Nat Biotechnol* 2004, **22**:595-599.
- Gill MS, Olsen A, Sampayo JN, Lithgow GJ: **An automated high-throughput assay for survival of the nematode *Caenorhabditis elegans*.** *Free Radic Biol Med* 2003, **35**:558-565.
- Tucker CL, Fields S: **Quantitative genome-wide analysis of yeast deletion strain sensitivities to oxidative and chemical stress.** *Comp Funct Genomics* 2004, **5**:216-224.
- 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.
- 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.
- 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.
- 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.
- Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, et al.: **A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*.** *Nature* 2000, **403**:623-627.
- 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.
- Shoemaker DD, Lashkari DA, Morris D, Mittmann M, Davis RW: **Quantitative phenotypic analysis of yeast deletion mutants using a highly parallel molecular bar-coding strategy.** *Nat Genet* 1996, **14**:450-456.
- 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.
- Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU, Findlay KA, Smith TE, Murphy MP, Bulter T, et al.: **A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity.** *Nature* 2001, **414**:212-216.
- Keller JJ, Giardiello FM: **Chemoprevention strategies using NSAIDs and COX-2 inhibitors.** *Cancer Biol Ther* 2003, **2**:S140-S149.
- Wilkinson DS, Pitot HC: **Inhibition of ribosomal ribonucleic acid maturation in Novikoff hepatoma cells by 5-fluorouracil and 5-fluorouridine.** *J Biol Chem* 1973, **248**:63-68.
- Mayo VS, De Kloet SR: **Effect of 5-fluorouracil on the synthesis and stability of ribosomal RNA in *Saccharomyces carlsbergensis*.** *Arch Biochem Biophys* 1972, **153**:508-514.
- Berger E: **The ribosomes of *Drosophila*. Normal and defective ribosome biosynthesis in *Drosophila* cell cultures.** *Mol Gen Genet* 1977, **155**:35-40.
- Ghoshal K, Jacob ST: **An alternative molecular mechanism of action of 5-fluorouracil, a potent anticancer drug.** *Biochem Pharmacol* 1997, **53**:1569-1575.
- Santi DV, McHenry CS: **5-Fluoro-2'-deoxyuridylate: covalent complex with thymidylate synthetase.** *Proc Natl Acad Sci USA* 1972, **69**:1855-1857.
- Mori H: **From the sequence to cell modeling: comprehensive functional genomics in *Escherichia coli*.** *J Biochem Mol Biol* 2004, **37**:83-92.
- Pratt RJ, Aramayo R: **Improving the efficiency of gene replacements in *Neurospora crassa*: a first step towards a large-scale functional genomics project.** *Fungal Genet Biol* 2002, **37**:56-71.
- Silva JM, Mizuno H, Brady A, Lucito R, Hannon GJ: **RNA interference microarrays: high-throughput loss-of-function genetics in mammalian cells.** *Proc Natl Acad Sci USA* 2004, **101**:6548-6552.
- Paddison PJ, Silva JM, Conklin DS, Schlabach M, Li M, Aruleba S, Balija V, O'Shaughnessy A, Gnoj L, Scobie K, et al.: **A resource for large-scale RNA-interference-based screens in mammals.** *Nature* 2004, **428**:427-431.
- Berns K, Hijmans EM, Mullenders J, Brummelkamp TR, Velds A, Heimerikx M, Kerkhoven RM, Madiredjo M, Nijkamp W, Weigelt B, et al.: **A large-scale RNAi screen in human cells identifies new components of the p53 pathway.** *Nature* 2004, **428**:431-437.