The biological magic behind the bullets

Brent R Stockwell

Testing the relative fitness of thousands of yeast strains helps determine pathways and proteins targeted by small-molecule drugs

A major challenge for researchers in the pharmaceutical industry and academicians is determining the mechanisms by which small organic chemicals, such as some drugs, affect living systems. Historically, such mechanism-of-action studies have taken years, if not decades, to reveal relevant proteins and pathways and there has been no systematic method or universal route for determining how drugs exert their biological effects. In recent years, however, a tool kit for facilitating such studies has been assembled that comprises several approaches including affinity and biochemical purifications, yeast three-hybrid analysis, protein-display methods and protein arrays[1]. In this issue, Parsons *et al.*[2] now extend this tool kit with a powerful new approach—a yeast-based global fitness test that reveals genes involved in mediating the response of yeast cells to a test compound.

The problem of determining proteins and pathways affected by low molecular weight organic compounds dates back to the turn of the 20th century, when Paul Ehrlich proposed that organic compounds affect living systems by interacting with specific molecular receptors (*e.g.*, proteins) in mammalian cells—specificity that could also be exploited in their development as therapeutic 'magic bullets.' It took the greater part of the 20th century for scientists to develop methods for identifying specific receptors for compounds of interest, but several notable successes over the past four decades have provided inspiration for the field.

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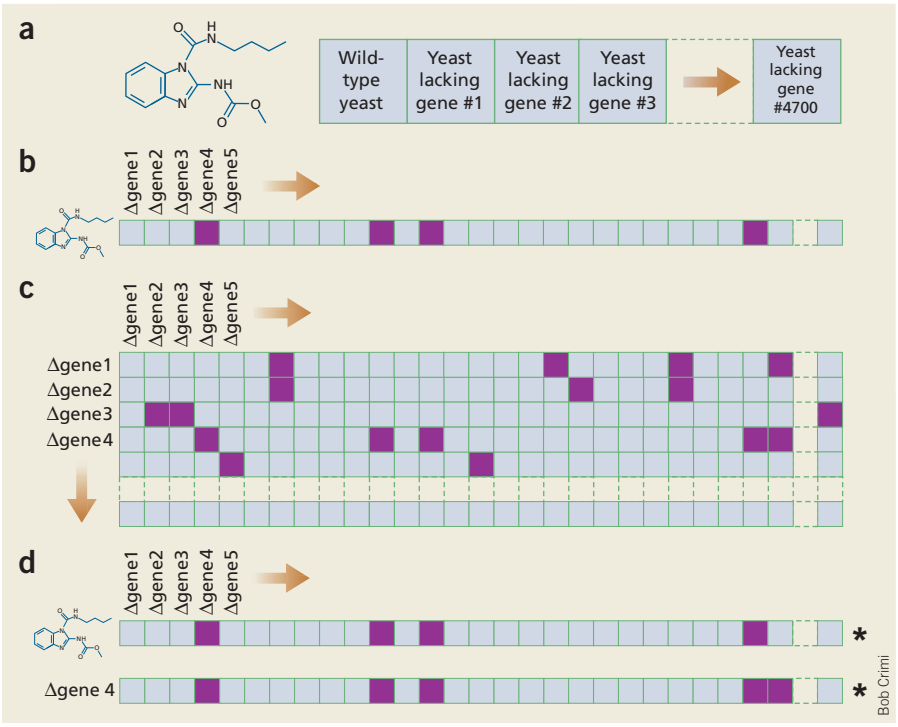

Figure 1 Schematic representation of the combined synthetic lethality/chemical screen for small molecules. (**a**) 4,700 yeast strains, each with a deletion in a different gene, are treated with the compound of interest. (**b**) Generation of the synthetic lethal profile showing a list of gene deletions that render cells hypersensitive to the test compound. (**c**) Generation of a synthetic lethal matrix listing all combinations of two genes that are lethal to cells. (**d**) Matching the synthetic lethal profile of the test compound with closest profile(s) of gene deletions provides an indication of possible mechanism of action.

Numerous compounds were used to discover important protein targets: colchicine for tubulin, steroids for steroid receptors, acetylcholine for the acetycholine receptor, histamine for the histamine receptors, FK506 for calcineurin and rapamycin for mTOR (mammalian target of rapamycin)[3]. These targets compose a veritable molecular pantheon of modern pharmacology and biology. Undeniably, determining the proteins targeted by biologically active compounds can also yield unexpected and important insights into the molecular determinants of living systems.

Most of these early investigations used a biochemical or affinity purification strategy. In this somewhat laborious procedure, analogs of a test compound harboring a radioactive isotope or a linker to a plastic bead are created. Target proteins are purified either by the bound radiolabel or by sticking to a bead-immobilized compound. The

limitation of this approach is that it is necessary to find ways to modify the structure of the test compound to introduce these requisite elements without interfering with biological activity. In many cases, this proves to be difficult or impossible, or the affinity between the test compound and the target protein is not sufficient for the approach to be successful.

Therefore, in recent years, several efforts have focused on developing technologies for determining a compound's macromolecular target that don't require modification of the compound's structure[4]. Innovations have allowed more rapid determination of protein targets of small molecules in some cases, but these methods frequently require structural modification of the test compound. Parsons *et al.* now demonstrate that the yeast-based global 'fitness test' can be a powerful approach for studying small molecules. Moreover, their strategy does not require structural alteration of the test compound.

In developing their strategy, the authors made use of synthetic lethal tests drawn from classical yeast genetics[5–7]. In a traditional yeast synthetic lethal screen, one searches for combinations of gene deletions or mutations that together cause cell death, but that individually have little or no effect on the organism. In this case, the term synthetic lethal is used to denote that the lethal event derives from the synthesis (*i.e.*, combining together) of two different deletions, rather than from a single deletion. Such synthetic lethal screens have been useful for identifying genetic interactions and mapping networks and pathways regulating cellular processes.

Parsons *et al.* apply this strategy of synthesizing genetic changes and chemical treatments to identify genes involved in mediating the response to chemical treatments. We would expect that if a small molecule disrupts the function of its target protein, then cells with less of that target protein would be more sensitive to the compound. Thus, by testing the sensitivity to a compound of a large number of yeast strains with defined gene deletions, we might expect that among the most sensitive strains would be a strain that harbors a deletion in the gene encoding the target protein[8]. Parsons *et al.* perform several proof-of-principle experiments to test this notion in their system and indeed discover that this phenomenon is sometimes observed. In one example, the authors treat a panel of 4,700 yeast strains[9,10], each with a different gene deletion, with the microtubule depolymerizing agent benomyl. They observe that yeast strains with deletions in genes involved in regulating cell structure, chromosome structure and mitosis are particularly sensitive to benomyl. Among these deletions is one encoding TUB3, a tubulin subunit of microtubules.

There is also a second strategy for using synthetic lethality to determine proteins and pathways affected by small molecules. In this second approach, one needs to generate a comprehensive set of yeast synthetic lethal 'profiles' by deleting a specific gene and testing the effects of deleting all other genes in combination with the test gene. This list of gene-gene synthetic lethal combinations is essentially a molecular fingerprint for each gene deletion, in that it lists other genes that are functionally linked to the test gene. Comparing such 'synthetic lethal profiles' for genes and compounds can be informative (see **Fig. 1**).

By comparing the synthetic lethal profile of a gene deletion to the synthetic lethal profile of a compound, one can determine whether they affect similar cellular processes. In an ideal world, we might expect that benomyl and a tubulin gene deletion would exhibit identical synthetic lethal profiles, unambiguously defining tubulin as the target of benomyl. In the real world, compounds have pleiotropic effects and can cause partial or complete loss of function with kinetics different from gene deletions. Thus, we should expect that a compound would share a similar, but perhaps not identical, synthetic lethal profile with a deletion in the gene encoding the protein targeted by the compound. Parsons *et al.* found that this real-world scenario is indeed the case. Benomyl and TUB2 have similar, but not identical, synthetic lethal profiles, as do cyclosporine A, FK506 and a calcineurin B deletion, which is a target of these compounds.

Synthetic lethal analysis in yeast is likely to be a useful new approach for studying small molecules. However, many compounds of interest act on proteins that are not expressed in yeast cells, so it will be necessary to extend this analysis to the 30,000+ genes found in each mammalian species. Although such a task is daunting, it is potentially feasible using large-scale collections of plasmids encoding short hairpin vectors that mediate knockdown of complementary mRNA sequences via the recently discovered process of RNA interference.

In summary, we can expect this and other chemical genetic approaches to bring insights into the mechanisms of action of existing drugs, investigational drugs and biologically active compounds. And, almost a century after Paul Ehrlich's seminal hypothesis, perhaps we can finally begin to demystify some of the magic behind the bullets.

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Cancer surgery joins the dots

Roger F Uren

Quantum dots show potential as a tool for imaging sentinel lymph nodes and guiding surgery.

Only the most experienced and adept surgeons can perform sentinel lymph node biopsy (SLNB), an increasingly popular strategy for determining whether a cancer has spread (metastasized). Some help may be at hand to guide the surgeon's scalpel in the form of a paper reported in this issue by Kim and colleagues[1], which demonstrates the visualization of sentinel lymph nodes in large animals via optical imaging of near-infrared (NIR) fluorescence emitted by quantum dots. The work represents not only a pioneering medical application of nanocrystals, but also a step forward in the clinical application of SLNB in cancer surgery.

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