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Lethal combinations

Chandra L Tucker & Stanley Fields

Synthetic lethality occurs when two otherwise nonlethal mutations together result in an inviable cell. A new study describes a rapid approach to identify synthetic lethal mutations in yeast.

When you drop your rowboat oar into the lake, you can still manage to paddle with the other one. But when you lose the second oar, you hope you're close to land. When the boat springs a leak, you can use a pump to bail it out. But when the pump breaks, it's time to find the lifejackets. Analogous logic is commonly exploited by geneticists, who seek mutations at additional loci that enhance or suppress the phenotype caused by a particular mutation. Two mutations are considered synthetically lethal if in combination they result in cell death, whereas either alone leads to a viable cell. Mechanistically, synthetic lethality can be due to two genes acting in parallel redundant pathways (as with the two oars). Alternatively, the two genes can act in the same, essential pathway, with the combination of the two effects being lethal (the leak and the broken pump).

The phenomenon of synthetic lethality was first described in *Drosophila melanogaster* by Bridges and later by Sturtevant and by Dobzhansky, who coined the term (discussed in ref. 1). But as with other genetic strategies that got their start in bigger organisms, it's been in the humble yeast *Saccharomyces cerevisiae* that this approach has reached its apogee. Numerous synthetic lethal screens have been carried out in yeast in the last decade through the use of a simple plasmid dependency assay[2]. Two years ago, Tong *et al.*[3] introduced a genome-wide approach that takes advantage of the collection of viable gene deletion mutants[4]. On page 277, Ooi *et al.*[5] describe a genomic strategy that should be a faster and more quantitative means to screen for synthetic effects.

Why should human geneticists be concerned with the intricacies of how thousands of yeast double mutants can be generated and analyzed? For one thing, the yeast interactions can identify new features of essential processes that are conserved in mammals. But perhaps more intriguingly, the yeast studies may help enhance our understanding of human genetic variation, which contributes significantly to our health and well-being. This variation means that the same allele can confer different disease burdens to different individuals[6]. Cancer susceptibility, for example, may be largely due to multiple mutations in multiple genes, each conferring a small effect[7]. Attempts to make sense of this variation can be guided by the experiences gained in simple model organisms.

Synthetic lethal screens go genomic

Previously, Tong *et al.*[3] developed synthetic genetic array (SGA) analysis (**Fig. 1a**), which crosses a yeast strain containing a deletion of interest to an array of ~4,700 deletion strains, a set of ordered strains in which each open reading frame is systematically replaced with a kanamycin cassette[4]. The diploid strains are subjected to sporulation and haploid selection, and synthetic effects are observed as absent or poor growth of strains on a plate.

Now, Ooi *et al.*[5] have developed a new method, synthetic lethality analyzed by microarray (SLAM; **Fig 1b**). SLAM also uses the yeast deletion set, but takes advantage of 'molecular bar codes'[8]—short, unique sequence tags that flank each deletion (the 'UPTAG' and the 'DOWNTAG'). Two sets of the 4,700 deletion strains are grown in parallel in single pools, which are transformed with either an integrative disruption fragment that knocks out the gene of interest or a control fragment that does not cause gene

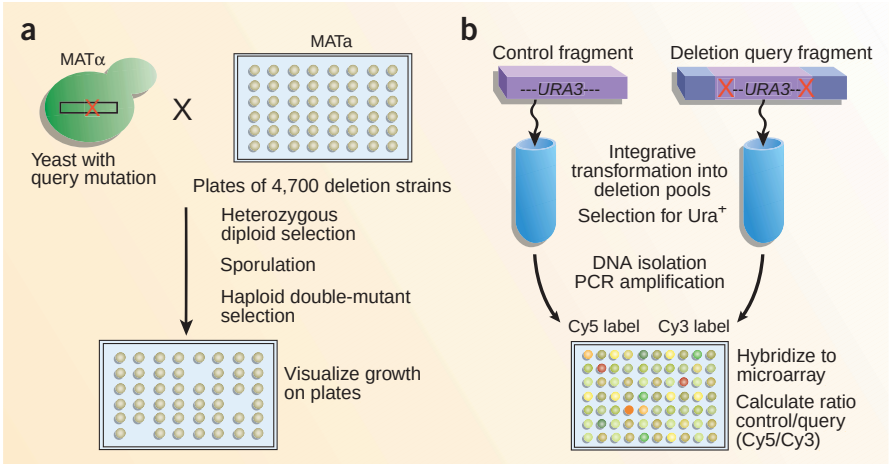

Figure 1 Comparison of genome-wide synthetic lethal screening methods. (**a**) Synthetic genetic array (SGA) analysis[3]. (**b**) Synthetic lethality analyzed by microarray (SLAM)[5].

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disruption. After selection for integration of the fragment and growth of the transformants, DNA is isolated from each pool and the tags are subjected to PCR amplification. Each tag is flanked by a common sequence, such that the same pair of PCR primers can amplify the tags for all 4,700 strains at once. The amplified DNA is hybridized to microarrayed oligonucleotides to quantify the growth of each strain. Synthetic lethal strains are identified by comparing control and deletion pool hybridizations.

An important advantage of SLAM as compared with SGA is reduced labor, as the deletion strains can be treated in pools instead of being monitored for growth individually. The double-mutant pools can be stored for additional assays under other selection conditions. One problem specific to SLAM, however, is that up to 15% of the integrative transformants may be incorrectly targeted in the genome. Another limitation is that only ~60% of the strains yield high-quality data for both UPTAG and DOWNTAG hybridizations, as a result of tag mutations, slow-growing strains or strains that are defective in integrative transformation. By comparison, the SGA method also shows problems with slow-growing strains and with strains defective in mating or sporulation. Limitations common to both methods are that they deal only with nonessential genes and analyze only complete deletions and not partial loss-of-function mutations.

Ooi *et al.*[5] characterized the synthetic lethal network of two genes that had also been analyzed by Tong *et al.*[3], allowing a direct comparison of the two methods. With the *SGS1* gene, each screen found a common set of synthetic interactors as well as ones that were not present in the other screen, implying that neither screen was saturating. SGA, which found 10 of 12 known synthetic lethal interactors with *SGS1* and 14 not previously identified, seems to be more sensitive than SLAM, which identified 7 of 12 known interactors and 5 new ones. False positives were a problem with both methods, to a similar degree (~50–60%), but these can largely be eliminated by further analysis.

Networks of synthetic genes

Both SGA and SLAM can potentially generate a large amount of data. An important challenge will be to develop ways to represent these data and to integrate them with results from other work, such as protein interaction or expression studies, to maximize the inferences that can be drawn about new genes or gene functions. One simple approach to visualizing large-scale synthetic lethal interactions has been the use of interaction maps, where a line is drawn between two colethal genes[3]. But these are genetic interactions, not physical ones, and as such represent many possibilities: genes with redundant functions, genes with additive effects on the same pathway or genes with indirect effects. These indirect effects can occur because a deletion phenotype represents not just the absence of one particular gene, but also the response of the cell to the absence of that gene, which may include upregulating or downregulating diverse pathways. If we can generalize from yeast[3,5], however, indirect effects may be rare, as most synthetic lethal interactions occur between genes involved in the same or similar processes.

Genomic methodologies for synthetic lethal studies are beginning to take shape in other organisms, in which techniques such as RNAi allow combinations of gene 'knockdowns' to be analyzed in worms, flies and even human cells. Thus, it becomes possible to envision screening a cell line mutant for a disease-related gene, such as a tumor suppressor, with a genome-wide array of RNAi constructs to search for synthetic effects. When the data from these types of screens emerge, it's likely that yeast will provide a guide for their analysis.

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Vibrating in the background

Alysson R Muotri & Fred H Gage

Retroviruses make up a large proportion of the mammalian genome. A new study shows that an mRNA nuclear export receptor can act as a modifier of endogenous retrovirus insertion mutations by interacting with the mutated pre-mRNA.

Mammalian genomes are notorious for hosting an incredible number of genetic parasites, known as transposable elements, which can interact with the surrounding genomic environment and increase the ability of the organism to evolve. Some of these mobile elements, called retrotransposons, are able to reproduce through an intermediate RNA, using the reverse transcriptase enzyme to insert a DNA copy in the genome in a new position. Humans and mice share ~40% of their genomes that is thought to have been derived from retrotransposons[1]. In humans, the activity of most of these parasites is believed to have been silenced about 40 million years ago (although we still have some of them jumping around in our cells), but mice have close to 3,000 active elements, responsible for 10–20% of spontaneous mutations[2]. The enormous contrast between the number of active elements in human and mouse suggests that the reason for the decline of transposon activity in humans may be related to some primary disparity between hominids and rodents[1].

Mouse intracisternal A-particles (IAPs) are retrotransposons similar to modern retroviruses but incapable of leaving the host cell owing to mutations in the envelope gene (*env*)[3]. These elements are severely repressed in most tissues of the mouse, possibly as a biological requisite for genomic stability and to reduce the transcriptional noise from pointless expression of RNAs. These observations suggest that the host genome has evolved effective epigenetic nuclear defenses that shield it from active retroelements, such as methylation and probably repressive chromatin structures. Consistent with this theory, homozygous DNA methyltransferase-1 (*Dmnt1*) knock-

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