PROTEOMICS

Live-cell map quest

A high-resolution interactome map that describes how proteins interact in living yeast cells is an invaluable reference for the research community.

"How does it actually look in the cell?" That is the question that Stephen Michnick of the University of Montreal wanted to answer about protein-interaction networks. In many methods typically used to screen for protein interactions or identify protein complexes, proteins are expressed in unnatural circumstances or cells are broken apart. To examine a situation closer to reality, Michnick and colleagues turned to a protein-fragment complementation assay (PCA) designed to study protein interactions in intact cells and expressed in their natural cellular compartments.

In a PCA, complementary fragments of a reporter enzyme are fused to proteins of interest; in this case, all the yeast proteins were 'of interest'. The reporter enzyme the researchers used was dihydrofolate reductase (DHFR). When two test proteins interact, the two DHFR fragments are brought together and fold to restore enzyme activity—which confers the ability to survive in selective medium. Michnick says they chose this life-or-death assay "because it is dead easy and very sensitive."

For the enormous undertaking of tagging all the proteins with the DHFR fragments, which Michnick referred to as "assembling a jumbo jet," a team of technicians used homologous recombination to insert cassettes encoding DHFR fragments into open reading frames. They did this in two *Saccharomyces cerevisiae* mating type strains, achieving 93% coverage of the ORFeome.

Then, with the aid of robotics for the binary interaction screen, they mated all of their fusion strains—two by two—on high-density arrays. In total, they performed more than 15 million matings and then used automated image analysis to identify interacting protein pairs.

In this broad survey the researchers identified 2,770 high-confidence interactions, 80% of which had not been previously discovered. They analyzed in detail interactions involving known and unknown protein complexes, and extended networks of protein complexes involved in autophagy and cell polarization. "What we saw in the complexes recapitulated a lot of what has been proposed, which is very satisfying, and there is an enormous amount of novelty in the network as well," notes Michnick, adding that since the publication of this work he has received feedback from researchers who identified previously unknown interactions within this dataset and are using this information to guide future research.

To improve the quality and quantity of information in the dataset, Michnick envisions approaches analogous to 'deep' sequencing—that is, probing subsets of the interactome in greater detail: "It's a lot harder to do, takes a lot more work and is now only practical at small scale." One such approach is to zoom in on protein complexes of interest, using the distance sensitivity of the PCA to describe the spatial relationships between subunits in complexes beyond the 8 nm in the current dataset. This can be accomplished by reducing the linker length in the fusions.

Another way to probe more deeply, covering more potential interactions, is to make all possible combinations of N- and C-terminal fragment fusions to increase the chance of creating functional constructs and detecting an interaction if there is one. "It's much easier to tag proteins at the C terminus," explains Michnick, "but in small-scale studies we found that if we test a known interaction via all possible combinations of N- and C-terminal tags, we will see it with at least one of those combinations. This kind of combinatorial screening would also improve coverage in [other] approaches as well."

Beyond a static interactome map, the PCA technology allows an assay of dynamic interactions, explains Michnick: "You can take our strains—which we are going to make readily available to the community—and replace the DHFR fragments with fragments of a reporter that would allow for a direct detection of signal, [such as] assays based on luciferases." PCA-based analyses of the interactome in mutants, in cells under various conditions as well as in cells treated with various compounds, will add yet another dimension to the protein-interaction maps.

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RESEARCH PAPERS
Tarassov, K. *et al.* An *in vivo* map of the yeast protein interactome. *Science*, published online 8 May 2008.