PROTEOMICS

Evolution of a screen

By forcing the interaction of proteins in a genetic screen in yeast, researchers can identify new players involved in biological processes.

Sometimes taking the old and giving it a few twists can yield surprising results. Michael DeVit, at the time in Stanley Fields' laboratory at the University of Washington, experienced this when he explored new ways to investigate the effects of protein-protein interactions. DeVit and Fields took the basic premise of a yeast two-hybrid screen—finding proteins that can bind to a bait protein—and reversed it: they forced proteins to bind to their bait and screened for those that activated a pathway of interest (DeVit *et al.*, 2005). This approach proved to be well-suited to dissect components of biological processes such as signal transduction.

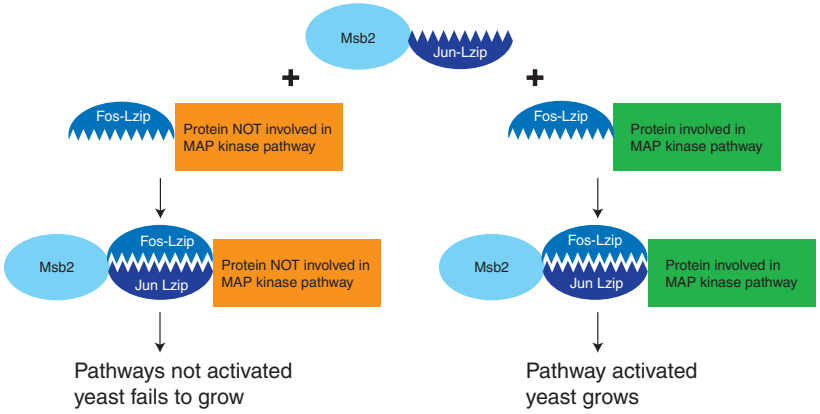

Figure 1 | Schematic of the assay. Msb2, a protein in the MAP kinase pathway, and library proteins, tagged with complementary leucine zippers (Jun or Fos-Lzip), are overexpressed in yeast. If a library protein is involved in the signaling pathway, its interaction with Msb2 can trigger signaling and growth; if a protein is not part of the pathway, interaction with Msb2 will not facilitate growth.

The first step in this assay, forcing the protein interaction, was the most technically challenging one. DeVit used the leucine zipper sequences of the transcriptional activators Jun and Fos, which are known to bind to each other with high affinity, and fused one to his bait and the other to his library of yeast proteins. In the first trial of the new system, he used Jun-GFP as bait and bound it to a library of Fos-proteins. By following the localization of GFP in the cell, DeVit could identify the intracellular destination of the target proteins. Although this provided the basis for a screen to find membrane-associated proteins, the real interest of DeVit and Fields was in identifying components of signal transduction pathways.

They focused their attention on a yeast MAP kinase pathway responsible for fila-

CHEMICAL TOOLS

FLUOROUS PROTEOMICS

It may not replace classical methods in proteomic applications, but fluorous-based chemistry provides a versatile and effective means of easily tagging and enriching a wide variety of peptide classes.

Most methods to detect post-translational modifications of proteins by mass spectrometry at the proteomic scale require enrichment of the proteins or peptides harboring the modification of interest before analysis. Many such techniques have been devised, with varying success. All of them, however, require the development of a reagent specific to that particular modification, considerably limiting the broad utility of each method.

At the Genomics Institute of the Novartis Research Foundation, researchers were looking for new ways to enrich samples for peptides containing modifications of interest. "We wanted to try something different than the traditionally employed fractionation methodologies—in effect, a more chromatographic or chemical approach to what biotin-strepavidin does," says Eric Peters, group leader of protein profiling/mass spectrometry. His group was familiar with a relatively new way of performing combinatorial chemistry, termed fluorous chemistry, wherein highly fluorinated chemical moieties are attached to small organic compounds to facilitate purification of the desired products of a chemical reaction. By exploiting the strong selectivity of fluorous-fluorous interactions, the labeled compounds will bind strongly and selectively to fluorous-functionalized silica gel, allowing their facile isolation from unlabeled compounds by washing the column with solvents consisting of varying compositions of water and methanol.

This fluorous approach has been used extensively for the targeted synthesis and purification of small molecules in organic solvents, but has never been used on aqueous biological samples. As Peters puts it, "We basically said, could you turn that thing around and start with an extremely complex mixture of biological origin and then pull out classes? We deal with peptides, and they are not small molecules—but could you do it? If you could, how well would it work?"

Much to their surprise, the fluorous approach actually works very well, as reported in the April issue of *Nature Biotechnology*.

mentous growth. DeVit transformed yeast with a fusion of Jun to Msb2, the initiator of the MAP kinase signaling cascade, and a library of Fos-labeled proteins (**Fig. 1**). Only library proteins that activated the pathway when interacting with Msb2 triggered signaling, which resulted in the growth of yeast; proteins that did not normally bind to Msb2 did not lead to signaling when the interaction was forced, and consequently the yeast did not grow. Using this screen, they identified a previously uncharacterized yeast protein to be involved in Msb2 signaling. Notably, it contained a peptide motif implicated in protein degradation, a finding that could add an interesting dimension to the regulation of the pathway and is now being investigated. Fields pointed out that this result underscored both the strength of the assay—the identification of new partners in a biological process—as well as its potential pitfalls. He cautioned: "Because of the artificial nature of the study, you cannot be sure that anything you come up with will be necessarily meaningful *in vivo*, so you have to carry out additional studies."

Taken as an initial screen for candidate proteins though, the applications of the assay are numerous. Asked for examples, Fields responded: "It would seem that for almost any process, such as signaling or transcription, DNA replication, protein transport and so forth, you can find a key protein that you put your zipper on, and then bring all other proteins to that site and look for phenotypes." He added, "Effectively the applications are limited only by the number of cell biological assays you can come up with." With only the imagination of scientists being the limit, this assay is likely to see wide use in exploring the intricate communication in protein networks.

Nicole Rusk

RESEARCH PAPERS
DeVit, M *et al*. Forcing interactions as a genetic screen to identify proteins that exert a defined activity. *Genome Res*. **15**, 560–565 (2005).

Peters' group demonstrated that they could use these fluorous affinity tags to enrich for peptides containing thiol or amino groups, or specific post-translational modifications such as phosphorylation. "We were actually shocked that it works as well as it does," says Peters. Not only does this allow different classes of peptides to be enriched using a simple solid phase extraction methodology, but the fluorous tags have other benefits for mass spectrometric analyses compared to biotin-based reagents, such as their inertness and possession of a mass defect. Furthermore, this method is equally applicable to metabolomics. To illustrate, Peters points out, "If you are looking at keto steroids, you wouldn't use a biotin derivative to isolate, efficiently recover and then analyze these species. However, you could use a fluorous hydrazide and pull out all the ketone containing species." Thus, this highly orthogonal way of separating biological compounds could be used in different fields of biology just as it has played a valuable role in many chemistry applications.

Daniel Evanko

RESEARCH PAPERS
Brittain, S.M. *et al.* Enrichment and analysis of peptide subsets using fluorous affinity tags and mass spectrometry. *Nat. Biotechnol.* **23**, 463–468 (2005).

NEWS IN BRIEF

PROTEOMICS

Quantitative mouse brain proteomics using culture-derived isotope tags as internal standards

One approach for proteomic analysis involves culturing cells with radiolabeled amino acids to generate a standard for mass spectrometric analysis of protein expression in cells growing under different conditions. Ishihama *et al*. modify this approach for use with biological samples, relative and absolute quantification of protein expression in animal tissues.

Ishihama, Y. *et al. Nat. Biotechnol.*; published online 17 April 2005.

RNA INTERFERENCE

Single-copy shRNA configuration for ubiquitous gene knockdown in mice

By specifically targeting transgene integration to the mouse *ROSA26* locus, it is possible to obtain efficient and ubiquitous RNA interference with a single-copy short hairpin RNA (shRNA). Seibler *et al.* demonstrate how this system can considerably simplify the production of genetic knockdown mice.

Seibler, J. *et al. Nucleic Acids Res.* **33**, e67 (2005).

CHEMICAL TOOLS

RNA structure analysis at single nucleotide resolution by selective 2′-hydroxyl acylation and primer extension (SHAPE)

Present chemical techniques for mapping RNA structure are limited by the need for multiple reagents. Merino *et al.* have identified a compound capable of reacting with the 2'-hydroxyl group of any ribonucleotide, subject to restraints imposed by RNA structure; by characterizing the pattern of modification, one can accurately analyze the folding of the RNA molecule.

Merino, E.J. *et al. J. Am. Chem. Soc.* **127**, 4223–4231 (2005).

CELL BIOLOGY

Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes

To better understand the functional importance of chromosome positioning within the nucleus, Bolzer *et al.* have used a three-dimensional fluorescent *in situ* hybridization approach that allows the differential labeling of all 24 chromosome types in human male fibroblasts. Using this technique, they create detailed position maps for both quiescent and actively cycling cells.

Bolzer, A. *et al. PLoS Biol.* **3**, e157 (2005).

CHEMICAL TOOLS

Four-color DNA sequencing by synthesis on a chip using photocleavable fluorescent nucleotides

Seo *et al.* designed four different nucleotides with a chemical cap and a photocleavable fluorescent moiety, for use in a 'sequencing by synthesis' strategy. Chip-bound DNA templates are subjected to a polymerase reaction in which each base must be uncapped before synthesis can continue, allowing controlled assembly and detection of fluorescence-encoded sequence data.

Seo, T.S. *et al. Proc. Natl. Acad. Sci. USA* **102**, 5926–5931 (2005).