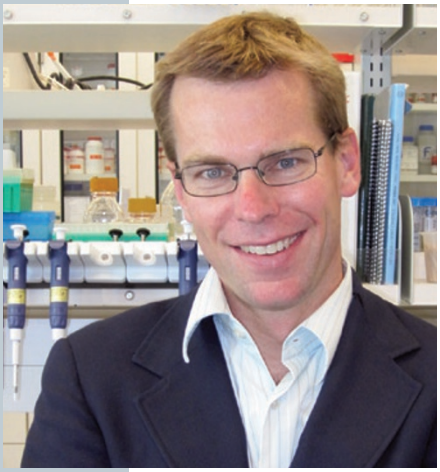
THE AUTHOR FILE

Trey Ideker

Yeast two-hybrid screens plus RNA interference assays are used to identify functionally relevant interactions.

Identifying protein-protein interactions and determining which ones are functional are often considered two separate pursuits, with the latter often requiring painstaking labor to sort out each interaction one by one. On page 801, Trey Ideker and colleagues present work in which they both find new protein-protein interactions and assess the functional relevance of these interactions.



In the mid 1990s, Trey Ideker, now at the University of California, San Diego, transitioned from designing guided missile systems to building protein networks. Ideker fell into graduate school after discontentment with his job threw him into the path of Leroy Hood at the University of Washington. Hood brought Ideker into the laboratory even though the latter had completed little coursework in biology. At the time, the first results of the Human Genome Project were just emerging, and the scientific community was beginning to realize that a list of gene sequences would not tell them everything they wanted to know. "It became apparent very early on that you couldn't build models of the cell without knowing what the protein interactions were," Ideker says. He was convinced that his computer-engineering skills could help, and, in fact, one of the papers he published as a graduate student helped establish the definition of systems biology as a field.

Ever since then, Ideker has integrated the disciplines of computer science and biology, building up models—dubbed 'interactomes'—of how numerous proteins interact in a cell. The last few years, he has worked to combine results of these interactomes, which attempt to identify proteins that make physical contact in cells, with results of RNA interference (RNAi) screens, which identify genes that affect phenotypes, such as cell growth or differentiation. "You go into a big hairball," he says, referring to the tangled appearance of interactome maps. "You find the RNAi targets [that came out of the screen] in the hairball and find out if they are connected," he explains.

But these kinds of studies rely on a previously generated network of protein interactions, and the proteins probed may not include those identified from RNAi screening. Ideker and his colleague Sumit Chanda, also at the University of California, San Diego, wanted to see whether they could turn the approach around, to generate a new protein network and interpret it using a targeted RNAi screen.

Ideker knew exactly which protein network he wanted to try. Several years before, Ideker had visited co-lead author Sudhir Sahasrabudhe, chief scientific officer of Prolexys Pharmaceuticals. The two had just finished a collaboration to map protein interactions in the parasite that causes malaria and had shown that this interactome was quite different from those in flies, humans and yeast. They began talking about how important the mitogen-activated protein kinase (MAPK) signaling cascade was and how no one had yet probed its interactions. They decided to launch a long series of yeast-two hybrid (Y2H) assays, in which they would modify important players of the signaling cascade as baits to fish out the proteins that might interact with them. Results of this study, Ideker now realized, could readily be used to design an RNAi screen with Chanda.

Using about 150 baits, Sahasrabudhe and Ideker identified over 2,000 interactions among nearly 1,500 proteins. From this, the researchers selected 14 proteins that had not previously been reported as interacting with the MAPK pathway. The researchers reasoned that they could validate these previously unreported interaction partners as functionally important, if knocking them down with RNAi affected the MAPK pathway (as assessed by expression of AP-1 or NFκB transcription factors, important effectors of the pathway). About one-third of the selected genes affected the transcription factors, providing strong evidence that the newly identified interaction partners were functionally important. "I was pleasantly surprised," says Ideker.

The result of this study led to the next collaboration, says Ideker. One of the functionally relevant interaction partners was the Na-H exchanger NHE1. The researchers sent an early draft of their manuscript to Diane Barber at the University of California, San Francisco, an expert on NHE1, who performed experiments indicating that NHE1 functioned as a MAPK scaffold, an important but previously unsuspected function.

The MAPK story will continue, Ideker says, and he is hopeful that the combined Y2H-RNAi approach will unleash a sea of stories from other scientists working on other pathways. "The beauty of this is that these are two highly automated technologies," Ideker says. "You have one robot that does your Y2H, and the other robot does RNAi. You turn the crank on these two technologies, and then the smarts come in on how you interpret the data."

Monya Baker

Bandyopadhyay, S. *et al.* A human MAP kinase interactome. *Nat. Methods* **7**, 801–805 (2010).