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New Functional Atlas Gives the 411 on Gene Partners

Sometimes it helps to have a cheat sheet when you are working on a problem as difficult as deciphering the relationships among hundreds of thousands of genes. At least that's the idea behind a powerful new technique developed by Howard Hughes Medical Institute (HHMI) researchers to analyze how genes function together inside cells.

The new approach is called epistatic miniarray profiles (E-MAP). The scientists who developed it — HHMI investigator Jonathan S. Weissman, HHMI postdoctoral fellow Sean Collins, and colleague Nevan Krogan, who are all at the University of California, San Francisco — have used E-MAP to unravel a key process that prevents DNA damage during cellular replication.

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In the best case scenario, E-MAPs are kind of like having the answers at the back of the textbook, said Weissman. You can look up a gene and get specific insights into what it's doing and which other genes it's doing it with.

Using the new technique, which enabled them to rapidly analyze more than 200,000 gene interactions, the researchers have made a discovery that helps explain how cells mark which sections of DNA have been replicated during cell division. If the marking process goes awry, DNA becomes damaged as it is copied.

Steps of this process were known before, but what wasn't known was which protein was actually carrying out the marking and how the mark was read, said Weissman, whose lab is at the University of California, San Francisco. Their work pinpointed a protein called Rtt109, which marks chromosomal components called histones, thereby signaling that a particular stretch of DNA has been replicated.

The E-MAP catalogs thousands of gene-gene (or epistatic) interactions in an entirely new way. Weissman's group, together with Krogan, developed the technique, which was instrumental in making the discovery reported in an advanced online publication in the journal *Nature* on February 21, 2007. Krogan carried out much of the work while he was a graduate student in Jack Greenblatt's lab at the University of Toronto.

E-MAPs provide information that's largely invisible to other types of intracellular studies, said Weissman. Comparing information from an E-MAP to other functional studies, like DNA microarrays or protein-protein interaction maps, is a bit like superimposing an X-ray over an ordinary photograph of a person so that you can get multiple views of how the different pieces fit together.

The discovery of Rtt109's role in protecting DNA is proof of principle that E-MAPs can help biologists quickly diagram basic cellular circuits, said Weissman. It's essentially a reverse engineering tool. Cells are very complicated systems, and we're trying to understand the underlying wiring. E-MAPs are a powerful tool to do that.

The key to E-MAPs is the ability to eliminate single genes or gene pairs and then analyze how each change impacts the growth of yeast colonies. Each yeast colony grows in a tiny spot on an agar plate, and each plate holds around 750 colonies. Software makes it possible to determine the growth rate of each colony and then compare the effect on growth of eliminating one gene at a time with the effect when two genes are simultaneously disabled.

The end result is a database that details the functional relationship of each gene to every other gene studied, revealing cases where the product of one gene depends on a second gene in order to carry out its cellular functions. In this experiment, Weissman's team looked at 743 yeast genes involved in basic chromosome biology. We wanted to look at everything that had to do with chromosome biology, including DNA replication, DNA repair, transcription to RNA, and so on, said Weissman. These are very basic cellular processes that are conserved from yeast to man.

To test their yeast E-MAP, Weissman, Collins, Krogan, and colleagues from the University of Toronto dissected the gene relationships of a large protein complex called Mediator, which helps regulate transcription of DNA into RNA. Researchers have painstakingly taken apart Mediator and put it back together again. It's been a huge amount of work, said Weissman. But with their yeast E-MAP, it was possible to quickly determine how the individual proteins that comprise Mediator interact with each other, as well as how they work with other proteins to turn genes on and off. We were able to dissect this large complex into subcomplexes that work together, he said. And so, in one fell swoop, we recapitulated many results about the Mediator complex which took years of careful work to obtain.

Weissman and his colleagues have made their findings from the yeast E-MAP freely available to other researchers, who can now mine the data to examine myriad more genetic relationships. The Interactome Database can be

found online at <http://interactome-cmp.ucsf.edu/>. We're working on making our Web tools more user friendly, to make it easier for others to analyze the data, said Weissman.

Weissman's team is now applying the E-MAP technique to other organisms. Ultimately, they're aiming to develop E-MAPs for humans.