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## Bridging the Gap

Aided by a mathematical tool for analyzing complex situations like shipping cargo and Internet traffic, Howard Hughes Medical Institute scientists and their collaborators at the Massachusetts Institute of Technology have mapped previously unknown pathways in yeast cells that link the overactivity of a mysterious gene to the death of nerve cells in Parkinson's disease.

If the findings translate to humans, they could shed light on the roots of the neurodegenerative disease and perhaps suggest molecular targets for new therapies, say the researchers, whose findings were published online February 22, 2009, in the journal *Nature Genetics*.

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Susan Lindquist, an HHMI investigator at the Whitehead Institute in Boston and a senior author of the report, says the mathematical recipe, or algorithm, used in the study will also help other researchers cut through confusing and often contradictory masses of data to home in on the truly important molecular connections that determine how cells respond to their environment and to their own pathologies.

Lindquist and her colleagues developed the approach to better understand how cells react to alpha-synuclein, a protein of unknown function that is found in Lewy Bodies, the hallmark pathological feature of Parkinson's disease. In those patients, the protein adopts a misfolded form that can build up in brain cells and become toxic.

Lindquist and her colleagues have been working to understand which signaling pathways connect the misfolding of alpha-synuclein to nerve cell death. As they began to accumulate data from various experiments, the group ran into a problem that Lindquist says is increasingly vexing in the era of high-throughput genetic analysis: different tools for finding functional connections yielded unrelated results. "It makes it hard to figure out what's going on," she said.

The conundrum is a bit like the parable of three blind men trying to describe an elephant by feeling different body parts – tail, trunk, and legs. Each man was biased by what he could feel, so that the resulting descriptions were

fragmented and contradictory, lacking the “big picture” context.

Similar discrepancies plague researchers when they search for pathways through which cells respond to changes in their environment. These types of studies are often done by manipulating individual genes – overactivating or deleting them, for example – and investigating how these changes alter cellular responses. A second workhorse method uses microarray technology to profile which genes are turned on or off when environmental conditions change.

The HHMI researchers found that in such experiments, the two data sets are sharply different, with little overlap. The reason is that each technique is biased toward finding certain kinds of genes – and not others. This makes it difficult to trace biologically relevant response pathways.

In studying alpha-synuclein toxicity, the Lindquist lab created 5,000 different strains of a yeast, *S. cerevisiae*, each containing a different overexpressed gene. All of the strains had also been modified to make toxic amounts of alpha-synuclein. The group noted which of the overactive genes had an effect on survival in the face of this excess alpha-synuclein.

To help in their search for gene pathways related to alpha-synuclein toxicity, the scientists used a vast collection of data on all the possible interactions between genes and regulatory proteins. “The problem is this collection of interactions – the so-called ‘interactome’ – is like a giant hairball where everything connects to everything else,” said Lindquist.”

Seeking a way to trim irrelevant parts of the “hairball” and focus on the most important connections, the Lindquist lab collaborated with Ernest Fraenkel’s lab in the Department of Biological Engineering at the Massachusetts Institute of Technology.

Fraenkel’s group harnessed a mathematical process that he says is “conceptually similar to linking up buyers and sellers or Internet users and determining the cheapest ways of connecting things.” In the case of alpha-synuclein, the scientists wanted to find not the cheapest connections, but the connections that were likely to be most crucial in the cells’ response.

The scientists tailored the algorithm to solve these kinds of problems, and named it “ResponseNet.” When they applied it to the yeast experiments, ResponseNet illuminated pathways whose manipulation altered cellular survival, and provided the first cellular map of the proteins and genes responding to alpha-synuclein expression.

“This algorithm provided new ways of thinking about the response to alpha-synuclein toxicity,” said Lindquist. “When the folks in my lab put them to the test experimentally, they worked. That told us that our way of connecting things up is working, and it gives us some promising ideas about therapy.”

Esti Yeger-Lotem, a postdoc in Fraenkel and Lindquist laboratories, said the method “allows for a more complete understanding of cellular response and can reveal hidden components of the response that may be targeted by drugs.”

For example, the algorithm revealed that a highly conserved pathway that can be manipulated with cholesterol-reducing statin drugs was involved in the toxicity of alpha-synuclein, as was another that can be influenced by the drug rapamycin.

ResponseNet will be a valuable addition to research on yeast, said Fraenkel, who intends to develop the method for use in mammalian systems. “If we can apply this to animals and humans,” he said, “it could open up a lot of valuable insights with relevance to therapy.”