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The plastic proteome

Researchers catalog how stresses affect the abundance and localization of every budding yeast protein.

Saccharomyces cerevisiae is a hardy species, able to survive in a variety of tough environments. By systematically tracking the levels and localization of proteins in individual cells, Breker et al. reveal how yeast dramatically remodel their proteome to adapt to different conditions (1).

Many studies have used microarrays or deep sequencing to map yeast cells' transcriptional responses to different stresses (2, 3). But, says Maya Schuldiner, from the Weizmann Institute of Science in Rehovot, Israel, changes in mRNA levels are only part of the stress response story. Protein levels are altered in a variety of ways besides transcription, and protein activity can be regulated by changes in subcellular localization. "Since proteins perform most cellular functions," Schuldiner explains, "we should really look at the proteins themselves to understand what is going on."

To do this, Schuldiner and her colleagues Michal Breker and Melissa Gymrek used a library of 5,530 yeast strains, each expressing a GFP-tagged version of a different protein (4). But to analyze so many strains in different stress conditions, Breker et al. needed to fully automate the process, from growing the cells to imaging them under the microscope. "It took us two years to build the system," Schuldiner says. "But now we can take pictures of 2,000 different proteins in one day. And we can screen the whole proteome in three days instead of months."

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Breker et al. monitored the abundance and localization of each GFP-tagged protein under normal growth conditions and in response to three different stresses: nitrogen starvation, oxidative stress induced by hydrogen peroxide, and reducing stress caused by dithiothreitol. Each condition provoked dramatic changes in protein levels, many of which couldn't be predicted from transcriptional analyses. "The studies on mRNA levels didn't capture the complete picture [of the stress response]," Schuldiner says. "There are enormous changes that occur at the protein level only."

Previous studies also missed the heterogeneity of cells' stress responses. Deep-sequencing and microarray techniques measure overall mRNA levels in a population of cells. But automated microscopy allowed Breker et al. to track protein abundance changes in single yeast cells, revealing that some proteins may be up- or down-regulated in response to the same environmental stress. The resulting "bimodal distribution," in which some cells produce more of a protein while others express less, helps budding yeast hedge their bets, in the hope that part of the population will be able to survive. Cells expressing high levels of the ribosome-associated chaperone Ssb1, for example, coped well when starved of nitrogen for 24 hours. But cells expressing lower amounts of Ssb1 were better at surviving starvation conditions that persisted for several days.

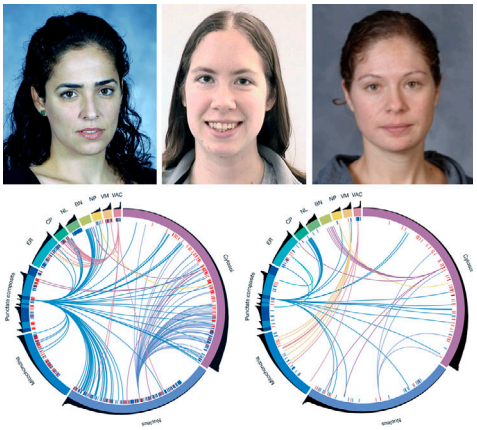
Breker et al.'s microscopy-based approach also revealed another aspect of the various stress responses, as many more proteins than expected showed a clear shift in localization. "Proteins' subcellular localizations are much more dynamic than we'd realized," Schuldiner says. "Hundreds of proteins can change their localization between very distinct compartments."

As expected, the three different stresses each induced different changes to the yeast proteome, but Schuldiner and colleagues were surprised at just how different these responses were. "We thought that there would be quite a lot of common changes, but only one group of proteins—those involved in forming P-bodies—consistently changed their localization in all three stresses," Schuldiner says. "This means we're only seeing the tip of the iceberg. If we look at other stresses, we'll probably find many more localization changes."

Breker et al. are now using their screening platform to examine how the proteome changes in different stages of the yeast life cycle and in response to other chemical and genetic perturbations. They're depositing their results in a database called LOQATE (5), which Schuldiner hopes will be widely used. "It's really simple to operate, even for people not used to handling enormous data sets," she says.

1. Breker, M., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201301120.
2. Causton, H.C., et al. 2001. *Mol. Biol. Cell*. 12:323–337.
3. Nagalakshmi, U., et al. 2008. *Science*. 320:1344–1349.
4. Huh, W.K., et al. 2003. *Nature*. 425:686–691.
5. Weizmann Institute of Science. Localization and quantitation atlas of the yeast proteome. http://www.weizmann.ac.il/molgen/loqate (accessed March 2013).

Focal Point



(Top row, left to right) Michal Breker, Melissa Gymrek, and Maya Schuldiner develop a single-cell screening platform to monitor how the abundance and localization of every yeast protein changes in response to different environmental stresses. Each stress induces a specific "thumbprint" of proteomic changes, as displayed for nitrogen starvation (bottom row, left) and oxidative stress (right). The researchers find that many changes in protein levels can't be explained by differences in gene transcription and that subcellular localization is much more dynamic than expected. Breker et al. also identify specific "bet-hedging" strategies that yeast use to survive adverse conditions.

BREKER AND SCHULDINER PHOTOS, COURTESY OF THE WEIZMANN INSTITUTE OF SCIENCE PHOTOGRAPHY DEPARTMENT; GYMREK PHOTO COURTESY OF THE AUTHOR