Being a control freak aids dividing cells

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Micromanagers may generate resentment in an office setting, but they get results in your body. New data indicate that a dividing cell takes micromanagement to the extreme, tagging more than 14,000 different sites on its proteins with phosphate, a molecule that typically serves as a signal for a variety of biological processes.

This preponderance of signals suggests that the cell may become a control freak during the division process, regulating each of its parts, no matter how obscure. It may take extreme measures to ensure that each "daughter" receives a full complement of cellular material. The new data—published online the week of July 28 in *PNAS*—open unexplored frontiers to developmental biologists, cancer researchers, and others who study cell growth and proliferation.

"There's a massive wave of phosphorylation in dividing cells, much bigger than anyone expected," says HMS associate professor of cell biology Steven Gygi, who is corresponding author on the study. "This discovery implies that we've severely underestimated the scope of regulation in cell division for decades, which has implications for our understanding of a wide-range of diseases and developmental defects linked to the cell cycle, from cancer to holes in the heart."

Traditionally, researchers probed cell division by zooming in on a particular gene or protein and tracing its interactions. But Gygi took a different approach. A leader in the emerging field of "proteomics," which involves looking at thousands of proteins at once, his team used an instrument called a mass spectrometer to essentially take a wide-angle

shot of dividing cells, capturing information that narrow studies missed. The panoramic view revealed a surprising level of signaling activity throughout the cell.

"An enormous number of proteins—more than 1,000—became highly phosphorylated during cell division, some more than 10 times," says postdoctoral researcher Noah Dephoure, who ran the experiment.

In collaboration with Chunshui Zhou, a researcher in HMS professor of genetics Stephen Elledge's lab, Dephoure worked with human cells, dividing them into two dishes. (The cells used are HeLa cells, which, while derived from a tumor, are used for many experiments because they thrive in culture. It's possible that some of the signaling events reported here are unique to these cells.) The first dish received nutrients with "heavy" carbon atoms—more massive than their "light" counterparts, which are abundant in nature. The second dish received normal nutrients, plus a toxic chemical to freeze the cells mid-division.

Dephoure and Zhou mixed all the cells together, killed them, chopped their constituent proteins—which were preserved—into small pieces called peptides, and fed these into a mass spectrometer. The instrument distinguished between otherwise identical peptides, based on the presence of "heavy" or "light" atoms, generating a ratio for each peptide. Dephoure paid particular attention to the ratios for peptides containing phosphate groups and uncovered major differences between the two populations of cells.

The dividing cells harbored a staggering number of regulated phosphate groups in unexpected places.

Gygi hypothesizes that the cell uses phosphorylation to break down every last protein complex before dividing. "Maybe the cell does something akin to putting Humpty Dumpty back together again at the end," he says.

"The massive number of phosphorylation changes in cell division strongly suggests that it involves a massive reorganization of the cell," adds HMS Department of Systems Biology chair Marc Kirschner, who was not involved in the study.

"Or the cell might phosphorylate everything to ensure that it hits a few key targets critical for proper division," says Dephoure. Under this scenario, extraneous phosphorylation may cloud the picture.

Armed with the team's list of proteins and phosphorylation sites, labs can conduct additional experiments to resolve this debate. They can investigate particular phosphorylation events and determine which ones contribute to successful regulation of cell division. Some may present therapeutic targets for patients with cell cycle diseases such as cancer.

"This study demonstrates how much a broad systematic approach to protein modification can facilitate experiments in the cell cycle field," says Kirschner. "We will be reaping results from this study for years ahead."

Source: Harvard Medical School