ERK's got rhythm: Protein that controls cell growth found to cycle in and out of cell nucleus (w/ Video)

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Time-lapsed video of individual breast tissue cells reveals a never-before-seen event in the life of a cell: a protein that cycles between two major compartments in the cell. The results give researchers a more complete view of the internal signals that cause breast tissue cells to grow, events that go awry in cancer and are targets of drug development.

The protein ERK, which helps cells respond to growth factors, travels back and forth between the nucleus, where genes are turned on and off, and the cell proper, where proteins work together to keep the cell functioning. In the video, individual cells pulsate with green light as an engineered fluorescent ERK fills the nucleus, exits and re-enters again in cycles that take about 15 minutes. The researchers don't know if the oscillation affects the activity of other proteins in a regulatory fashion, they report in December 1 issue of *Molecular Systems Biology*, but find the oscillations to be regular and robust.

"True oscillations in biology are rare," said lead author Steve Wiley, chief biologist at EMSL, located at the Department of Energy's Pacific Northwest National Laboratory. "And that the oscillations of such a major growth regulator could go undiscovered for so long is extremely surprising."

ERK As Anchor

One possible function of the oscillations could be in regulating how ERK interacts with other proteins. Regardless of its biological function, ERK oscillations between compartments represent a new behavior that proteins can exhibit within cells.

Biomedical researchers need an accurate mathematical model in hand to test anti-cancer drugs. Adding ERK oscillations into the model allowed Wiley's group to make better predictions about how breast cells will respond to changes in their environment, such as the presence of growth factors or cancer drugs.

"Current models used in drug development behave very differently from the model we came up with," said Wiley. "The oscillations anchored our model in reality."

Round and Round

The meaning of the handful of oscillations found by researchers within cells has been controversial. Calcium levels cycle up and down in nerve cells, but scientists still debate why 20 years after the discovery. The production and destruction of the well-known cancer-related protein p53 continuously cycles, but its purpose is unclear. ERK is one protein in a long chain of command involved in cell growth. Because ERK gets repeatedly activated and deactivated by various proteins, Wiley and colleagues thought it might oscillate.

ERK has a role in human breast tissue, where the molecule epidermal growth factor, or EGF, sends a message from the cell surface to the rest of the cell in a carefully regulated manner that includes ERK. In breast cancer, that chain of command goes awry and cells grow out of control. Cancer drug researchers target players in the chain of command to control that growth.

For that reason, Wiley and his colleagues wanted to better understand EGF's chain of command, also known as its signaling pathway. Most researchers use cancer cells, which are easy to manipulate in culture, but Wiley studied healthy breast tissue to find out what goes on in normal cells. In addition, most research examines the population of cells on average, in which individual differences between cells can get lost. This work watched single cells.

Green Eggs

Researchers know a lot about what activates ERK and what shuts it down in the EGF signaling pathway. To follow ERK, the scientists engineered healthy, cultured breast cells to produce a green-glowing version of the protein. When EGF turns on the signaling pathway, the team verified that the green version of ERK is activated in the same way as the regular version is, by the addition of a chemical group. Other proteins deactivate ERK by removing the chemical group, and the process repeats.

To see what happens in the cells, the team put the culture dishes under a microscope that took pictures automatically once a minute. Then they removed EGF from the cells' culture and let them settle in and quiet down. The cells looked like fried eggs awash in light green.

When the team returned EGF to the culture dishes, the nucleus within cells -- what looks like an egg yolk -- brightened up with green, indicating the ERK proteins were flooding into the nucleus. After a few minutes, the green drained from the nucleus back into the cell proper, only to return again after some time. The oscillations in individual cells cycled about every 15 minutes, starting out in sync but losing that coordination over time.

Also, the time-lapse video showed that cell reproduction didn't seem to affect the cycling. The oscillations continued regularly throughout cell growth. Then the oscillations briefly stopped while one cell divided into two daughter cells. As cell division finished up, the oscillations resumed.

Additional experiments showed the oscillations required EGF in the cell culture and continued for up to ten hours, the longest period of time the researchers observed. In addition, the number of cells with oscillating ERK depended on how crowded the living conditions were. For example, the team found that at the lowest numbers of cells, all of them showed oscillating ERK. As the cells reproduced, fewer cells oscillated. By the time the cells filled the whole surface of their dish, virtually all of the cells lost their cycling ERK.

A Model ERK

One way scientists determine how well they understand the workings of a cell is to see if they can simulate it in a computer program. Wiley and his colleagues developed such a model that included the oscillating ERK, as well as most of the players in the chain of command from EGF receptor on.

To test the model, the team first used the model to predict how the oscillations would behave under conditions that kept ERK activated for a prolonged period of time. The model predicted the oscillations would die out if ERK stayed on. When the team performed a biochemical experiment in which they prevented ERK from de-activating, the percentage of cells oscillating dropped off, as the model predicted.

To further explore the biological significance of the ERK cycles, Wiley's group would like to test whether other growth factors cause ERK to oscillate in breast cells and whether different types of cells exhibit the same sort of oscillations. Ultimately, they would like to know if they can tweak the oscillation to see how things change inside the cell.

More information: Harish Shankaran, Danielle L. Ippolito, William B. Chrisler, Haluk Resat, Nikki Bollinger, Lee K. Opresko and H. Steven Wiley, Rapid and Sustained Nuclear-Cytoplasmic ERK Oscillations Induced by Epidermal Growth Factor, Mol Syst Biol, DOI:10.1038/msb.2009.90

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