

Fluorescent-Probe in Worm Creates Real-Time 'View' of Cellular Stress

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Scientists at the University at Buffalo have created a mutant worm that changes color when it moves.

The color change is generated by an optical sensor called stFRET. The sensor is composed of a pair of fluorescent molecules connected by a molecular spring that is inserted into structural proteins in the worm's cells.

When the worm is prodded, stretching the structural proteins in muscle fibers, the linking spring is stretched, and the worm fluoresces in a different color. The color change is observable using a confocal microscope.

The fluorescence indicates the amount of mechanical stress in the host protein and this can be imaged in different parts of a cell or an organism. This development opens the door to studying in real-time pathological processes that are influenced by changes in mechanical stress, such as cardiac arrhythmias, muscular dystrophy and brain tumors.

"Mechanical forces are part of the life cycle of all cells whether they are protozoa, morning glories or ballet dancers," said Frederick Sachs, Ph.D., SUNY Distinguished Professor and senior author on the paper describing this development.

The study is published in the June 2008 issue of FEBS Journal, the journal of the Federation of European Biochemical Societies. The study

and images can be downloaded at www.blackwell-synergy.com/toc/ejb/275/12 . Sachs is a member of the Center for Single Molecule Biophysics in the Department of Physiology and Biophysics, UB School of Medicine and Biomedical Sciences, where the research is continuing.

"Muscles are the prototypical stress generators," said Sachs, "and hair cells in the inner ear are the prototype mechanical sensors, but all cells have elaborate networks of mechanical and chemical interaction. To figure out how mechanical stress produces its effects, you need to be able to measure the stress in known proteins as a function of time and space."

Scientists have long estimated the average stress produced by muscle or other cells, and more recently they have measured the stress from isolated molecular motor proteins, but intact cells are much more complex. To study this network of mechanical stresses and biochemical communication, termed the "mechanosome," requires specific probes that can respond rapidly to help separate cause from effect, noted Sachs.

To study the mechanosome, Fanjie Meng, a doctoral student in Sachs' laboratory, developed the optical sensor that can be inserted into the genes of a cell or an organism.

"After incorporating the probe into specific structural proteins like spectrin, actinin or collagen, we can observe the stress in these proteins when the cell moves by itself or when we pull or push it externally," said Sachs. "To date we have made mutant tissue-cultured cells as well as worms.

"It isn't hard to imagine making mutant animals that change color when they move, allowing scientists to study normal physiology and pathology," he added. "Imagine beating hearts with different protein

labels. We could map the distribution of mechanical stress in and around the heart from beat to beat."

The researchers currently are working to make the probe more sensitive, calibrating it for specific stresses, and synthesizing physically smaller probes so they have less impact on the host protein. Using these probes, they are applying defined mechanical stress to cells and animals and measuring how specific proteins respond. Thomas M. Suchyna, Ph.D., research assistant professor in the Center for Single Cell Biophysics, was a major contributor to this research, which is supported by a grant to Sachs from the National Institutes of Health.

Source: University at Buffalo

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