Mapping subcellular temperature profiles with genetically-encoded thermosensors

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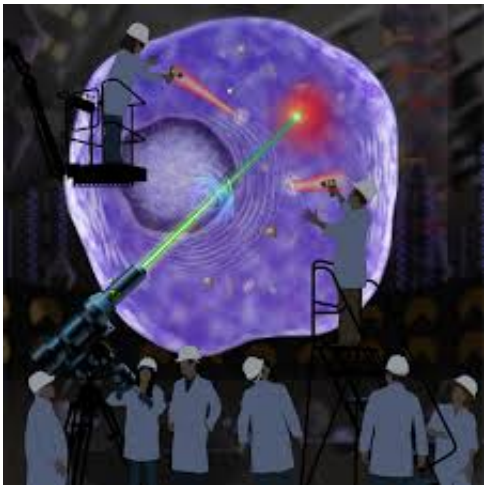

Measuring temperaure inside cells. Credit: Steven H. Lee

(Phys.org) —If you asked a biologist what any given cell is going to do next, they might ask you first to tell them its electrical potential, oxygenation, pH, osmolarity or glucose concentration. Depending on how finely-scaled your answer might be, they might be able to predict anything from firing an action potential or entering mitosis, to undergoing apoptosis. But what if you knew the subcellular temperature profile in such detail that each mitochondria, centriole and even regions of the endoplasmic reticulum could be read as easily as a mother her child's fever? That question now drives some of the most exciting research in biology. This year several groups have come up with ingenious thermometric methods ranging from fluorescence lifetime

microscopy to exotic diamond nanosensors employing nitrogen vacancy centers. The latest breakthrough, just published in *Nature Methods*, describes a method that uses a genetically-encoded sensor built from green fluorescent protein (GFP) that is fused to a thermosensing protein borrowed from Salmonella. The researchers used this construct to probe thermogenesis in brown fat mitochondria, and perhaps most astoundingly, were able to correlate temperature with both mitochondrial membrane potential and ATP production.

Infrared thermography is generally not suitable for imaging fine features in cells. Its spatial resolution is greater than 100μm and it detects only the surface temperature of the object. To measure organelle temperatures, you need a sensor that operates on their scale. A quantum dot or nanodiamond sensor perhaps 100nm wide could be incorporated into a cell by endocytosis, but once inside, it can go anywhere. These sensors can measure intracellular fluctuations of just 0.05 Kelvin (and outside the cell 0.0018), but unless they remain right at heat producing structures in the cell, they will probably only sense uniform thermal background. The reason for this is that not only does any heat generated by these organelles diffuse away rapidly, the organelles themselves like to move. It is worth mentioning that a fascinating paper appeared not long ago on the Arxiv server which attempted to measure how far the effects of the hydrolysis of a single ATP molecule could be felt. The number they came up with is the same size as the sensors we just mentioned, 100nm.

The first direct links between organelle function and temperature were made earlier this year by researchers from Japan. They developed a fluorescence lifetime method which used a polymer based thermometer. The temperature resolution they obtained was 0.2 C (apologies for the unit mixing) while the spatial resolution was diffraction limited. These researchers were able to show that the nucleus and centrosome were significantly hotter than other parts of the cell. We generally think of

cilia and their associated centrioles as being chemically or molecularlly controlled. Watching their response as a function of temperature may cast their primal role in a new light. While these studies are nice, what sets apart the new research (also done by a Japanese team), is that they could genetically encode a protein-based sensor, target it directly to different organelles like the mitochondria, and then simultaneously measure membrane potential and energy production to correlate temperature increases to the intrinsic function of the organelle.

The key to building this new sensor is a protein known as TlpA, which is made by Salmonella. The normal operation of TlpA is as an autoregulatory repressor that senses temperature to control transcription. At around 37 degrees C, it undergoes rapid and reversible structural transition to an unfolded monomer. By fusing the fluorophor region of GFP to TlpA, the researchers were able to make the fluorescence spectrum of GFP temperature dependent. The final step was to join the fusion protein to sequences that could target the protein to either the mitochondria, endoplasmic reticulum (ER) or plasma membrane.

The researchers were then able to simultaneously image the temperature-sensitive GFP along with the mitochondrial membrane potential indicator dye, JC-1. They found that that elevations in temperature and potential were highly correlated. Perhaps that is not so surprising, but it is the nature and order of these correlations where potency might be derived. They also confirmed this link using another genetically-encoded sensor (ATeam26), along with fluorescence resonance imaging (FRET)to measure ATP. Since ATP is synthesized primarily by an electrochemical pump during oxidative phosphorylation, its production should reflect the mitochondrial proton gradient as seen by JC-1.

The characteristics of these complex sensors were vetted by studying the well-known phenomenon of thermogenesis. The researchers looked at brown fat adipocytes, and also avian skeletal muscle, which is the

preferred method of heat generation in bird species. The full power of this technology will arguably best be brought to bear when it is applied to the brain. The thermodynamics of spiking neurons has been driven experimentally mainly by rather imprecise external temperature sensors. With a better handle on temperature changes, not just inside and outside axons, but inside the glial cells which myelinate them—and also carry a significant fraction of the spike energy, possibly as phase transitins—a better understand of neural transmission may be had.

Where neurons connect, the role of presynaptic mitochondria as transactors of electro-mechanical spike currency into chemo-mechanical vesicle dynamics might now be imaged in telling detail. Similarly on the opposed side of the synapse, where mitochondria are scuttled as a function of minipotential to sop or source calcium, ATP and heat itself, their broader function as hidden communicator of information and power not just at the level of synapse, but cell, may be revealed.

More information: Genetically encoded fluorescent thermosensors visualize subcellular thermoregulation in living cells, *Nature Methods* (2013) DOI: 10.1038/nmeth.2690

Abstract
In mammals and birds, thermoregulation to conserve body temperature is vital to life. Multiple mechanisms of thermogeneration have been proposed, localized in different subcellular organelles. However, visualizing thermogenesis directly in intact organelles has been challenging. Here we have developed genetically encoded, GFP-based thermosensors (tsGFPs) that enable visualization of thermogenesis in discrete organelles in living cells. In tsGFPs, a tandem formation of coiled-coil structures of the Salmonella thermosensing protein TlpA transmits conformational changes to GFP to convert temperature changes into visible and quantifiable fluorescence changes. Specific targeting of tsGFPs enables visualization of thermogenesis in the

mitochondria of brown adipocytes and the endoplasmic reticulum of myotubes. In HeLa cells, tsGFP targeted to mitochondria reveals heterogeneity in thermogenesis that correlates with the electrochemical gradient. Thus, tsGFPs are powerful tools to noninvasively assess thermogenesis in living cells.