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Fluorescent sensors reveal subcellular thermal changes

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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, studying thermogenesis directly in intact organelles has been challenging. Visualizing patterns of thermal changes at subcellular resolution would reveal physiologically relevant spatio-temporal information, especially if this could be done in the native cellular configuration of the cell. Here we review and compare the wide variety of intracellular thermosensors currently identified. This review focuses particularly on genetically encoded sensors. It also explores the notable physiological discoveries made using these imaging methods, which are rapidly becoming indispensible to the study of thermal biology.

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Introduction

Endothermic species such as humans employ multiple strategies to maintain thermal homeostasis. They produce heat by sustaining an active metabolism and by sacrificing fuel efficiency. Some of these mechanisms increase ATP hydrolysis and are related to metabolic cycles and maintenance of the cationic gradient across membranes in liver, muscle, and brown adipocytes. Others derive heat from the proton-motive force of aerobic electron-transport *via* a regulated proton leak in the mitochondria of brown adipose tissue [1–3]. Obtaining data describing the patterns of thermal changes at subcellular resolution in the native cellular configuration could reveal physiologically relevant spatio-temporal information.

Earlier studies of conventional thermometers utilized infrared (IR) techniques. IR thermography is non-invasive and has reasonably good temperature resolution, but only moderate spatial resolution (up to 10 μm). Furthermore, it can only monitor the cell surface [4]. Recently, detection of heat production from a single cell was reported by a mechanical microresonator with a sensitivity of 1 mW [5], but these techniques are also unable to measure intracellular temperatures and inside of the cell mass. In order to observe intracellular temperature distribution, the molecular thermometers should simultaneously satisfy multiple requirements: high temperature resolution; high spatial resolution; functional independence from environmental changes in pH, ionic strength, and surrounding biomacromolecules; and a concentration-independent output. Notably, the absence of any of these features obstructs intracellular temperature mapping [6••]. Fluorescent thermosensors have attracted much attention because they allow direct measurement of intracellular temperature. They are also superior to contact thermometers in monitoring temperatures within the micro-/nanometer range because there is no interference from the medium between the probe and the evaluated object.

Here, we will discuss the wide scope of intracellular thermometers and their features (summarized in Table 1), and explore some of the interesting physiological discoveries made using these sensors.

Organic and inorganic material-based thermosensors

Inorganic material based thermosensors

To date, several thermal sensors based on inorganic materials have been reported. One approach uses the temperature-dependent phosphorescence intensity of the rare earth chelate Eu-TTA (europium (III) thenoyltrifluoro-acetonate) [7–10,11•]. This thermosensitive dye was used to image intracellular heat waves evoked in Chinese hamster ovary cells after activation of the metabotropic m1-muscarinic receptor [7]. Fast application of acetylcholine onto the cells evoked a biphasic heat wave that was blocked by atropine, and after a brief delay was followed by a Ca^{2+} wave. Atropine alone produced a monophasic heat wave in the same cells, suggesting that its interaction with the receptor activates some intracellular metabolic pathways. This approach, however, does not have the spatial resolution to monitor subcellular events [7].

Quantum dots are another example of inorganic material-based thermosensors [12–14]. They exhibit superior brightness for detection, and are resistant to pH and other

Table 1

Reported thermosensing techniques for single cells

Year	Last author	Read out mode	Material	Category	Ref
1998	Yoshioka	Phosphorescence intensity	Eu-TTA	Inorganic	[7]
2004	Ishiwata	Phosphorescence intensity	Eu-TTA	Inorganic	[8]
2007	Ishiwata	Fluorescence intensity	Eu-TTA	Inorganic	[9]
2012	Suzuki	Fluorescence intensity	Eu-TTA	Inorganic	[10]
2014	Suzuki	Fluorescence ratio	Eu-TTA	Inorganic	[11•]
2010	Capobianco	Fluorescence intensity	Er^{3+}	Inorganic	[13]
2010	García Solé	Fluorescence intensity	Quantum dot	Inorganic	[12]
2011	Lin	Fluorescence spectrum	Quantum dot	Inorganic	[14]
2013	Lukin	Fluorescence intensity	Nanodiamond	Inorganic	[17••]
2008	Koutsogeorgis	Fluorescence decay rate	La_2O_2S:Eu and La_2O_2S:Tb	Inorganic	[36]
2009	Weissleder	Luminescence	Y_2O_3 nanoparticles	Inorganic	[37]
2013	Nienhaus	Fluorescence intensity	Gold particle	Inorganic	[38•]
1995	Tromberg	Fluorescence decay time	NBD, laurdan	Organic	[39]
2009	Uchiyama	Fluorescence intensity	Synthetic polymer	Organic	[15]
2012	Uchiyama	Fluorescence life time	Synthetic polymer	Organic	[6••]
2013	Uchiyama	Fluorescence life time	Synthetic polymer	Organic	[16]
2011	Hernandez	Fluorescence intensity	β-Galactosidase activity	Genetically encoded	[40]
2012	Quidant	Fluorescence anisotropy	GFP	Genetically encoded	[26]
2013	Quidant	Fluorescence anisotropy	GFP	Genetically encoded	[27••]
2013	Mori	Fluorescence ratio	Engineered GFP	Genetically encoded	[25••]

environmental variations that are expected to affect intracellular conditions. The spectroscopic characteristics of quantum dots have been shown to be a strong function of temperature both at the bulk and at the single-particle levels. Maestro *et al.* have reported the characterization of quantum dot spectral shifts dependent on intracellular temperature changes in cells under two-photon excitation [12], whereas Vetrone *et al.* have used Er/Yb-doped nanocrystals to look at intracellular temperature in HeLa cells [13]. More recently, a complete experiment and analysis of time-dependent localized intracellular temperature responses following Ca^{2+} stress and physical cold shock was presented, representing the first experimental evidence of non-homogeneous local temperature progression in cells [14].

Synthetic polymer based thermosensors

Synthetic polymer-based thermosensors have also been reported [6••,15,16]. A highly hydrophilic fluorescent nanogel thermometer was maintained in the cytoplasm and emitted strong fluorescence even at high temperatures. Thus, intracellular temperature variations associated with biological processes can be monitored by this novel thermometer with a temperature resolution of better than 0.5°C [15].

This approach was expanded for intracellular temperature mapping, in which the authors took advantage of temperature-dependent changes in fluorescence life-time to visualize heat production from mitochondria observed as a proximal local temperature increase. The results suggest that this new synthetic polymer-based thermometry may be useful in defining the intrinsic relationship between temperature and organelle function [6••].

Nanodiamond thermometry

The third approach uses coherent manipulation of the electronic spin associated with nitrogen–vacancy color centers in diamond. The technique enables the detection of small temperature variations in an ultrapure bulk diamond sample. Using nitrogen–vacancy centers in nanodiamonds, the local thermal environment on length scales as short as 200 nm can be measured directly. To demonstrate the feasibility of using this technique in living cells, these nanodiamonds and gold nanoparticles were introduced into WS1 cells using nanowire-assisted delivery. Temperature variation was measured at a single nanodiamond while slightly heating the gold nanoparticles in two different locations, without significant damage to the cells. Thus, by introducing both nanodiamonds and gold nanoparticles into a single human embryonic fibroblast, the control of temperature gradients and subcellular temperature mapping were demonstrated [17••].

These methods have sufficient spatial resolution to make them compatible with monitoring subcellular events in single cells, but the introduction of thermosensor molecules into cells is invasive, involving microinjection or electroporation. Furthermore, there is currently no way of subcellular targeting of these molecules to specific organelles within the cells. Genetically encoded sensors may represent a method to overcome this problem.

Genetically encoded thermosensors

Genetically encoded biosensors based on fluorescent proteins such as green fluorescent protein (GFP) have a number of features that make them attractive as *in vivo* reporters [18]. In contrast to adding chemical probes exogenously, these sensors can be explicitly targeted to