Form 3

Abstract of Thesis

Name （VU CONG QUANG）	
Title	Development of genetically encoded temperature indicators for intracellular thermometry at the subcellular level (細胞以下レベルの温度計測のための遺伝子的にコードされた温度指示薬の開発)

Abstract of Thesis

Genetically encoded temperature indicators (GETIs) allow the measurement of temperature dynamics at a subcellular resolution in live cells. However, GETIs have suffered from low temperature sensitivity when comparing to other nanothermometers, e.g., chemical-based fluorescent nanothermometers. To clearly visualize heat production from a biological process, it is highly desirable to develop GETIs that exhibit high temperature sensitivity for accurate temperature measurement as well as high specificity to temperature. In this thesis, I present two GETIs: (1) **E**lastin-**L**ike **P**olypeptide based **TEMP**erature indicator (**ELP-TEMP**) with the highest ever temperature sensitivity among the existing fluorescent nanothermometers, and (2) **B**lue-excited **g**enetically encoded **TEMP**erature indicator (**B-gTEMP**) that responded specifically to the temperature. ELP-TEMP is comprised of a temperature-responsive elastin-like polypeptide (ELP) fused with a cyan fluorescent protein (FP), mTurquoise2 (mT), and a yellow FP, mVenus (mV), as the donor and acceptor, respectively, of Förster resonance energy transfer (FRET). At elevated temperatures, the ELP moiety in ELP-TEMP undergoes a liquid-liquid phase transition leading to an increase in the FRET efficiency. In HeLa cells, ELP-TEMP responded to the temperature from 33 to 40 °C with a maximum temperature sensitivity of 45.1 ± 8.1 percent signal change per one degree Celsius (%/°C), which was the highest ever temperature sensitivity among the existing fluorescent nanothermometers. Although ELP-TEMP showed sensitivity not only to temperature but also to macromolecular crowding and self-concentration, I was able to correct the output of ELP-TEMP to achieve accurate temperature measurements at a subcellular resolution. I successfully applied ELP-TEMP to measure temperature changes in live cells induced by a local heat spot, even if the temperature difference was as small as <1°C, and to visualize heat production from Ca^{2+} influx induced by a chemical stimulation. Furthermore, I investigated temperatures in the nucleus and cytoplasm of live HeLa cells and found that their temperatures were almost the same within the temperature resolution of the measurement. This result would provide important information to shed light on a controversy about a discrepancy between a theoretical calculation and experimental measurements of intracellular temperature changes in single cells. On the other hand, B-gTEMP is a chimera of a green FP, mNeonGreen (mNG), showing low temperature sensitivity of −0.7%/°C, and a red FP, tdTomato (tdT), showing the highest temperature sensitivity of −2.9%/°C among the examined FPs. B-gTEMP is the improved version of gTEMP, a GETI composed of a blue FP, Sirius, and a green FP, mT-Sapphire (mT-Sap). B-gTEMP showed a response in a wide temperature range of 15–50 °C with an average temperature sensitivity of 2.2 ± 1.2%/°C, comparable to that of gTEMP (2.6%/°C). Because B-gTEMP utilized a visible light excitation whereas gTEMP utilized ultraviolet excitation, temperature imaging with B-gTEMP showed lower phototoxicity and autofluorescence background than that of gTEMP. Additionally, B-gTEMP showed higher temperature resolution than gTEMP. Furthermore, temperature measurement with B-gTEMP was not affected by macromolecular crowding and self-concentration, which are the advantages over ELP-TEMP. Using B-gTEMP, I successfully monitored quick temperature rises induced by a local heat spot with a temporal resolution of 10 ms. In addition, I demonstrated the functionality of B-gTEMP in conventional temperature imaging to measure heat production in mitochondria induced by a chemical stimulation, and investigated temperature in the nucleus and cytoplasm of live HeLa cells, and found that the temperature was almost the same between them within the temperature resolution of the measurement, consistent with the result of ELP-TEMP. Altogether, ELP-TEMP and B-gTEMP are useful GETIs for future investigation of cell thermobiology.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

遺伝子にコードされた蛍光性温度指示薬は、生細胞中の動的な熱現象をサブ細胞レベルで可視化可能であり、温度生物学研究の有用なツールとして期待されている。しかし、従来の蛍光性温度指示薬は、測定感度や温度特異性に問題があった。Vu氏は、温度変化に対して急峻に構造変化を起こし下限臨界溶液温度を示すエラスチン類似ポリペプチドを温度感受性ドメインに用いることを考案し、従来に例がなく高感度な蛍光性温度指示薬ELP-TEMPの開発に成功した。ELP-TEMPの蛍光シグナルは温度以外の外因からも影響を受けて測定誤差を生じる問題があったが、Vu氏はELP-TEMPの安定発現細胞株の樹立と、細胞中のELP-TEMPの自己濃度や分子クラウディングを考慮した校正を行うことでその問題を解決し、高精度な細胞内温度計測法を確立した。

本論文において、その専門知識と研究能力によって優れた発想と問題解決を行い、温度生物学研究への貢献が期待される研究成果を得た。以上により、博士の学位を授与するに相応しいと判断した。