論文の内容の要旨

論文題目 **Visualization of SERCA pump activity in living cells with a novel FRET-based conformation sensor**

和訳 **新規 FRET 構造センサーによる生細胞における SERCA ポンプ活性の可視化**

指導教員 坂井　克之　准教授

東京大学大学院医学系研究科

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脳神経医学専攻

氏名　佐藤　嘉名与

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Intracellular Ca^{2+} plays a central role in controlling numerous cellular processes such as exocytosis, gene transcription, cell proliferation, muscle contraction and cell survival. The versatile spatiotemporal Ca^{2+} dynamics has been considered to be responsible for the regulation of such diverse cellular functions. The level of intracellular Ca^{2+} is determined by the balance between the influx that introduces Ca^{2+} into the cytoplasm and the efflux that removes it from the cytoplasm. The key molecules involved in the regulation of intracellular Ca^{2+} such as channels, pumps and exchangers have been identified. Channels in the plasma membrane and sarco/endoplasmic reticulum (SR/ER) membrane are responsible for the Ca^{2+} influx, while pumps and exchangers carry out the Ca^{2+} efflux.

Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is a Ca^{2+} pump that transfers Ca^{2+} from the cytosol to the lumen of the SR/ER at the expense of ATP hydrolysis. The role of SERCA is pivotal for maintaining intracellular Ca^{2+} homeostasis and determining the spatiotemporal pattern of Ca^{2+} signals. Indeed, impairment of SERCA causes Ca^{2+} homeostatic dysfunction resulting in several important disease states such as heart failure, hypertension, diabetes, cancer, Alzheimer's disease and psychiatric disorder.

The sequential conformational changes of SERCA are accompanied by active transport of 2 moles of Ca^{2+} across membranes per 1 mole of bound ATP; the conformational changes of SERCA are directly linked with its activity. Since the three-dimensional structures of the different conformational states have been solved, the detailed mechanism of the reaction cycle of SERCA is known. Traditionally, SERCA pump activity has been approximated by a simple Michaelis-Menten equation as a function of the cytosolic Ca^{2+} concentration with a Hill coefficient of 2. Almost all the theoretical models for intracellular Ca^{2+} dynamics use this approximation. However, it remains to be elucidated whether the actual enzymatic activity of SERCA pumps in living cells is identical to that of previous *in vitro* and dynamics of SERCA activity in living cells is still unknown.

In this study, we addressed this issue by applying an optical technique to monitor SERCA activity in living cells. We have developed a FRET-based SERCA2a activity sensor, designated F-L577, which retains the ATP-dependent Ca^{2+} pump activity.

We demonstrated that the FRET efficiency between donor and acceptor of F-L577 was dependent on the conformational state of the molecule by state fix experiment and confirmed that the FRET signal changes of F-L577 directly reflect the instantaneous Ca^{2+} pump activity in living cells by ER luminal Ca^{2+} imaging. Using F-L577, we succeeded in visualizing the conformational changes of SERCA2a that accompany ATP-dependent Ca^{2+} transport. Dual imaging of cytosolic Ca^{2+} and the FRET signals of F-L577 in living cells revealed that SERCA2a activity is coincident with the oscillatory cytosolic Ca^{2+} concentration changes evoked by ATP stimulation. These results comprise the first experimental evidence for oscillatory SERCA activity synchronized with Ca^{2+} oscillation in living cells. Further, the SERCA dynamics measured by whole cell imaging revealed that, the activity of SERCA2a in living cells can be expressed by a function of the cytosolic Ca^{2+} concentration. Notably, in my experimental system, the Hill coefficient was estimated to be 6.8, rather than 2 (the value expected given 2 Ca^{2+} binding sites). The cooperative dependence of Ca^{2+} pumps on cytosolic Ca^{2+} is an important parameter for the generation of complex patterns of Ca^{2+} signals, such as Ca^{2+} oscillations, because small changes in this parameter have a large impact on the behavior of Ca^{2+} dynamics in theoretical models. As found for the action potential, which is generated by the combination of opposite membrane potential changes promoted by fast voltage-gated sodium channels and slowly activated voltage-gated potassium channels, cytosolic Ca^{2+} spikes may be generated by the ingenious balance of Ca^{2+} influx and Ca^{2+} efflux promoted by Ca^{2+}-release channels and Ca^{2+} pumps,

respectively. Therefore, revealed high cooperativity provide us with evidence to reconsider not only the SERCA pump activity but also the Ca^{2+} release activity in living cells for better understanding of the mechanism underlying the generation of Ca^{2+} dynamics.

Our new findings indicate that the activity of SERCA2a is markedly dependent on the cytosolic Ca^{2+} concentration, and that SERCA2a acts as a rapid switch to refill Ca^{2+} stores efficiently in living cells for shaping the intracellular Ca^{2+} dynamics and for reducing its cytotoxicity. The F-L577 protein constructed in this study will be useful for future studies on Ca^{2+} signaling in normal and abnormal cellular processes that involve SERCA pump activity and provide significant insight into the regulatory mechanism of SERCA activity in living cells.