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Protein ligand-tethered synthetic calcium indicator for localization control and spatiotemporal calcium imaging in plant cells

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ABSTRACT

In plant biology, calcium ions are involved in a variety of intriguing biological phenomena as a secondary messenger. However, most conventional calcium indicators are not applicable for plant cells because of the difficulty with their localization control in plant cells. We here introduce a method to monitor spatiotemporal Ca^{2+} dynamics in living plant cells based on linking the synthetic calcium indicator Calcium Green-1 to a natural product-based protein ligand. In a proof-of-concept study using cultured BY-2 cells overexpressing the target protein for the ligand, the ligand-tethered probe accumulated in the cytosol and nucleus, and enabled real-time monitoring of the cytosolic and nucleus Ca^{2+} dynamics under the physiological condition. The present strategy using ligand-tethered fluorescent sensors may be successfully applied to reveal the spatiotemporal dynamics of calcium ions in living plant cells.

Ca^{2+} ion is one of the most important second messenger in cell biology.[1,2] A variety of synthetic or genetically encoded calcium indicators have been developed to reveal the spatiotemporal dynamics of calcium in a living cell, and various calcium-related signaling pathways have been elucidated through monitoring dynamic changes in calcium concentration.[3–5] Conventional calcium indicators have been developed for applications with mammalian cells, but the majority are unsuitable for plant cells.

In plant cells, a luminescence-type calcium sensor, aequorin, is commonly used as the most reliable sensor.[6–8] Although several signaling pathways have been monitored in the presence of aequorin in model plants, genetic transformation of the cells is essential for the use of such a protein-based sensor. Thus, various intriguing Ca^{2+}-associated biological phenomena in non-model plants, such as tendril coiling,[9,10] trap-closure of Venus flytrap,[11,12] and nyctinasty in legumes,[13] have not been investigated because of the infeasibility of genetic transformation. On the other hand, useful synthetic calcium indicators are limited in number because they must be able to penetrate the cell wall. In addition, it is extremely difficult to apply a small-molecule calcium indicator to monitor cytosolic Ca^{2+} in the plant cell. This is because a small-molecule calcium indicator that penetrates the cell wall will be further trapped in the vacuole, in which the Ca^{2+} concentration is much higher (0.2–5 mM) than that of the cytosol (100 nM).[14] Therefore, for broad application with non-model plants in which genetic transformation is presently infeasible, useful synthetic calcium indicators that localize in the cytosol are highly desirable.

We developed a ligand-tethered calcium indicator suitable for applications in plant cell biology by using a natural product-based ligand (Scheme 1). To evaluate our ligand-tethered calcium indicator, we used tobacco (*Nicotiana tabacum*) BY-2 cells for proof of concept. This probe showed efficient cell permeability and localized within the cytosol and nucleus by means of ligand–protein interaction, and thus enabled spatiotemporal monitoring of Ca^{2+} ions in the BY-2 cells.

Localization of conventional calcium probes in the plant cell: In previous Letters, the emission-ratio-type calcium probe Indo-1 was reported to be the only synthetic calcium indicator useful for plant cells.[15–17] However, its application requires a specially tailored protocol called acid-loading. In this protocol, the cells are incubated in acidic medium (pH 4.5) containing a high concentration of Indo-1 (50 μM) for 1 h, in order that the cytosol and nucleus are stained with Indo-1. In the present experiment, Indo-1 was confirmed to localize in the cytosol and nucleus of BY-2 cells, but the cells were indicated to be physiologically damaged (Fig. 1a). The acid-loaded BY-2 cells were not stained with fluorescein diacetate (FDA) as an indicator of cell viability (Fig. 1a–c and e), which implied that the acid-loading protocol caused serious damage to the plant cells.[18,19]

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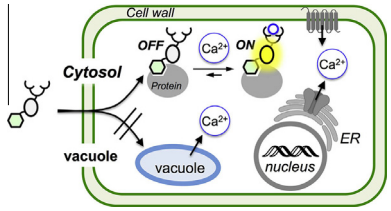

Scheme 1. Schematic illustration of ligand-tethered calcium indicator for control of cell permeability and intracellular localization in plant cells.

Acetoxymethyl (AM) esterification of the Ca^{2+} chelater (BAPTA) is a standard technique to confer efficient cell permeability to the indicators in mammalian cells. However, Indo-1-AM did not stain the contents of cells in the culture medium, indicating that Indo-1-AM showed low cell permeability, possibly because of its hydrophobicity or low esterase activity within the BY-2 cells (Fig. 1d). Fluo-4-AM, a widely used calcium indicator, also yielded no fluorescent signal in BY-2 cells (Fig. 2a). In addition, Fura2-AM and Calcium Green-1-AM mainly localized in the vacuole under the same condition (Fig. 2b and c). These results indicated that almost all commonly used calcium indicators fail to localize in

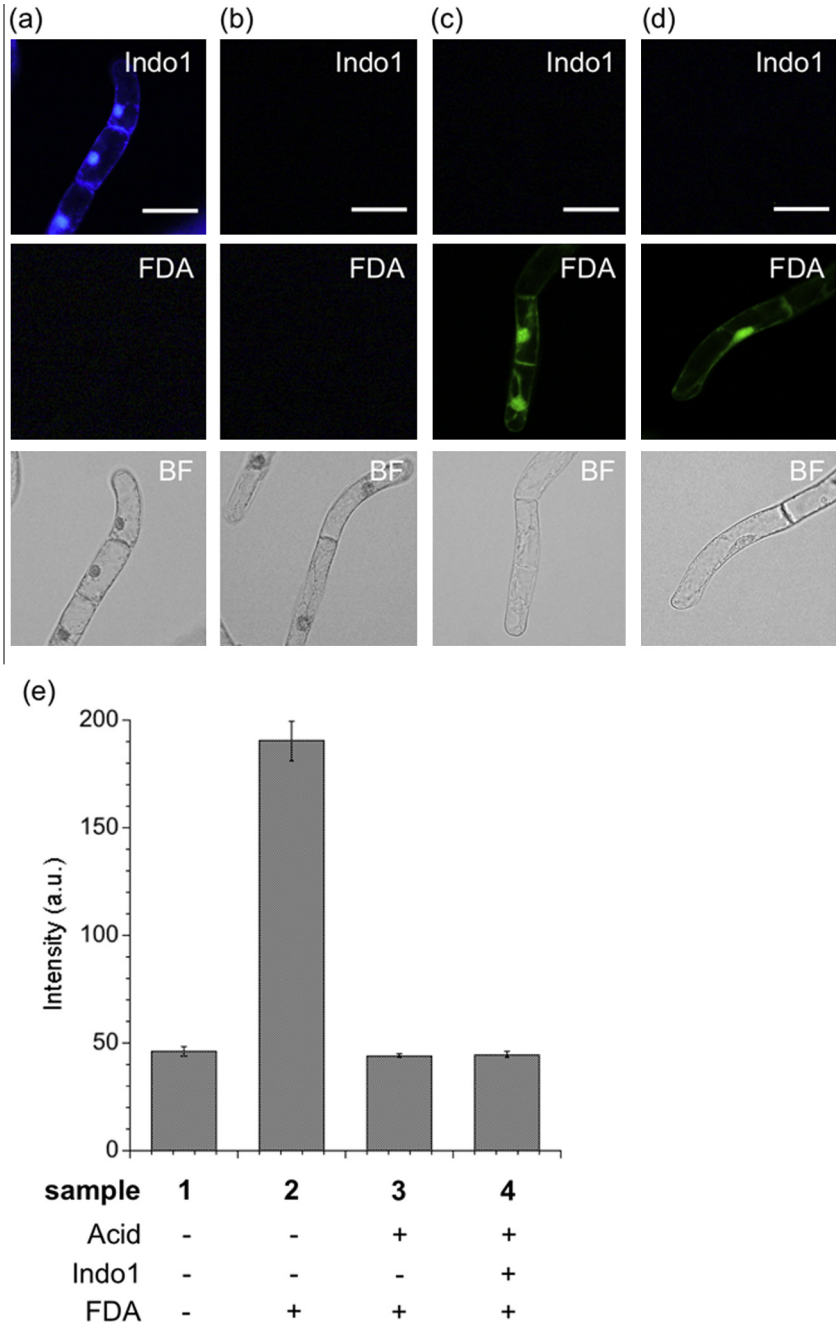

Figure 1. Fluorescent signal of BY-2 cells stained by fluorescein diacetate (FDA) and Indo-1. Fluorescent microscopic images of BY-2 cells stained by FDA and Indo1-free (50 μM) in the acidic condition (a), by FDA only in the acidic condition (b), by FDA only in culture medium (c), and by FDA and Indo1-AM (50 μM) in culture medium (d). The top images show Indo1-derived fluorescence, the middle images show FDA-derived fluorescence, and the bottom images show bright-field images. Scale bar 50 μm. (e) Intensity of FDA-derived fluorescence in Indo-1-stained BY-2 cells with or without acid-loading.