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Genetically Encoded Biosensor to Know Plant Behaviour

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SUMMARY

The plant cell is a hub of tremendous biological activities. The dynamics of intracellular biomolecules inside the plant can also be influenced by various biotic and abiotic stresses. The quantification of these intracellular biomolecules is challenging. Biosensor is a powerful tool for monitoring the dynamics of biomolecules. This approach was initially limited to small number of substrates for which specific dyes were available. However, with the development of genetically encoded biosensors, now it has become possible to study large number of biomolecules at cellular level. Genetically encoded biosensors, introduced into cells through transgenic approaches, offer the benefits of quantitative imaging. The number of biosensors for different biomolecules are increasing. This offers an opportunity of applying those biosensors for optical imaging of important biomolecules. The genetically encoded biosensors are designed by many companies which have wider applications in plants. Some of the applications are metabolite imaging, imaging of ROS, redox conditions, calcium imaging, pH analysis, phytohormones, nutrients, etc.

INTRODUCTION

The use of genetically encoded biosensors in plant biology was initially slow compared with other fields, because of technical problems associated with the imaging of plant cells. However, continuous efforts from multiple laboratories in the past decade have overcome a number of initial difficulties, and some genetically encoded sensors have become a standard laboratory tool. Genetically encoded biosensors are used as the preferred method to visualize and analyse ion fluxes, signalling components, and metabolites, covering an expanding palette of cellular processes. While fluorescent proteins as such are mainly used for localization and expression studies, genetically encoded fluorescent biosensors in addition allow real time studies of cell metabolism with a similar high spatial and temporal resolution (Gjetting *et al*.,2013). Cell-specific promoters allow biosensor expression in the target cell of choice in contrast to chemical probes that are inherently dependent on efficient delivery into the cells.

Types of Genetically Encoded Biosensors

Single Fluorescent Protein (Single-FP) sensors

The sensing mechanism in single fluorescent protein based sensor is within the fluorescent protein itself, such as pHluorins, or where sensing is coupled to a ligand binding domain. Other options using single fluorescent proteins include protein-protein interactions reported by fluorescent protein reconstitution (biFC) or detection of protein translocation. One notable exception of specific plant relevance is the DII-Venus auxin sensor, where degradation of the fluorescent protein is utilized as sensing mechanism.

Fluorescence Resonance Energy Transfer (FRET) sensors

The ligand binding causes a conformational change of the sensor leading to a change in FRET ratio between two fluorescent proteins, usually CFP/YFP variants. Usually, FRET sensors consist of a FRET donor-acceptor pair and a ligand-binding domain. The conformational change in the ligand-binding domain is revealed by the change of FRET efficiency between the attached donor and acceptor molecules. Since FRET is extremely sensitive to both the distance and dipole-dipole orientation between the fluorophores, change in FRET efficiency can sometimes be observed in a configuration in which conformational change in the ligand-binding domain does not seem to induce any distance change between the donor and acceptor (Okumoto, 2012). In case of FRET the donor is excited and the emission of donor and acceptor recorded. The ratio between these two measurements gives a measure of FRET efficiency and so of the conformation of the sensor domain. Such ratio analysis comes with the added benefit of being highly quantitatively reliable, correcting for many optical artefacts and for differences in expression level and reporter localization (Hilleary *et al*., 2018). Due to this fact, a large number of ligand-binding domains have been successfully converted into FRET sensors.

Methodology for Development of GE Biosensor

Vector construction and transformation

The construct should be design by adding coding region for promoter, restriction sites, fluorescent proteins, ligand and sensor domains, localization signal, and terminator. After construction it has to transfer into *E. coli* and then in *Agrobacterium* for transformation. The Agrobacterium mediated transformation method can be followed.

Expression analysis using RT-PCR

For the confirmation of transformation, the total RNA has to be isolate and convert into cDNA. The cDNA is use for RT-PCR reaction with gene-specific primers.

In vivo imaging using Fluorescence Microscope

The fragments of young fibrous roots of 10 days old seedlings were separated and immobilised on coverslips (Fisher Scientific, USA) using low-melting-point agarose (Amresco, USA), and the parts of the root were exposed. The coverslips were put in an Attofluor® Cell Chamber (Invitrogen) to form a perfusion chamber. The prepare slide keep under fluorescence microscopy and visualise using appropriate wavelength.

Analysis Using Software

All fluorescence images were collected and briefly processed using MetaFluor software (Molecular Devices), and then the resulting data were further analysed using Matlab R2012b software and plotted using Prism 5 software.

Applications of Genetically Modified Biosensors

Many of the sensors created for mammalian cells are not extensively used in plants. This is partly because of the differences in the intracellular biomolecules in plants and animals. Some cellular processes for which genetically encoded sensors are extensively used in mammalian cells do not operate the same way in plants (e.g. protein relocalisation with the production of specific phosphatidylinositol species and extensive G-protein signaling). However, there are a few exceptions where biosensors provided new insights into as yet undiscovered cellular processes in plant cells (Walia *et al*., 2018). Some of the applications such as metabolite imaging, imaging of ROS, redox conditions, calcium imaging, pH analysis, phytohormones, nutrients, *etc.* are described.

Metabolite Imaging

In the past decade, a large array of genetically encoded sensors for metabolites has been developed. Fluorescent Indicator Protein (FLIP) family sensors, pioneered by the Frommer lab, utilized periplasmic binding proteins (PBPs) from Gram-negative bacteria. The PBP family contains members recognizing a wide variety of substrates including central metabolites such as sugars and amino acids, and various ions such as phosphate and sulfate. The PBPs undergo a large conformational change when bound to their respective ligands, making lucrative scaffolds for FRET-based sensors. Various metabolite sensors for various substrates including maltose, glucose, ribose, sucrose, arabinose, glutamate, glutamine, histidine, and leucine/isoleucine/valine have been developed using PBPs as the ligand-recognition domains (Okumoto *et al*., 2012).

Imaging of Reactive Oxygen Species (ROS)

Traditionally, various dyes such as dihydrodichloro fluorescein diacetate (H_2DCF-DA, for H_2O_2) and nitroblue tetrazolium (NBT, for the superoxide radical) have been used to visualize different species of ROS in plant cells (Swanson *et al*., 2011). However, many of these chemicals need to be loaded into the cells, making the measurement difficult, and have some problems with photo-oxidation. Hyper, consisting of a bacterial peroxide sensor (OxyR) and cpYFP is a H_2O_2-specific genetically encoded sensor. The two redox-active cysteine residues in Hyper are located in a hydrophobic pocket of the OxyR domain, hence they are accessible only to amphiphilic molecules such as H_2O_2 (i.e. unlike roGFPs described below, Hyper does not respond to Oxidized form of

glutathione (GSSG) in vitro; (Belousov *et al.*, 2006). Hyper was successfully used to monitor H_2O_2 levels in the cytosol and peroxisome of tobacco and Arabidopsis.

Calcium Imaging

Genetically Encoded Calcium Indicators (GECIs) have been targeted to several subcellular compartments and membranes, with recent targeting of YC3.6 and YC4.6 to the chloroplast stroma (Loro *et al*., 2016). Targeting of GECIs into organelles may facilitate discovery of organelle-specific calcium responses. By contrast, the attachment of GECIs to the cytoplasmic side of compartment-specific membranes may increase the spatial resolution of calcium response analyses (Wagner *et al*., 2015).

pH Analysis

The pH homeostasis is important for secondary transport processes, protein modifications and sorting, and vesicle trafficking. Intracellular pH gradients are established through the coordinated activity of H^+ pumps and associated ion transporters and are indispensable for cellular compartmentalization and ion homeostasis (Schumacher, 2014). Early work was performed using ratiometric pHluorin, optimized for plants (termed phGFP) (Moseyko and Feldman, 2001). phGFP enabled the visualization of pH gradients in Arabidopsis roots, with more acidic pH (6.5-7) in root-cap cells, relatively alkaline pH (7.3-7.6) in the elongation zone, and intermediate pH (7-7.3) in the meristematic zone. Using ratiometric pHluorin researchers reported pH gradients and pH oscillations in tobacco pollen tubes. Cytoplasmic pH oscillations in root hairs were also observed by using the pH-sensitive GFP (H148D). Ratiometric pHluorin has also been used to estimate apoplastic pH and a whole palette of targeted ratiometric pHluorin variants has been used recently to map the pH in various subcellular compartments and organelles (Martiniere *et al*. 2013).

CONCLUSION

The success of these genetically encoded biosensors suggests that most of the biological, chemical or molecular events can be monitored. By this approach the development and deployment of biosensors constitute an iterative process. As more precise biosensors are yet to be deployed in plants, the contribution of genetically encoded biosensors to plant biology stands to grow in the future.

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