Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Sense and sensibility: the use of fluorescent protein-based genetically encoded biosensors in plants

Richard Hilleary[1], Won-Gyu Choi[2], Su-Hwa Kim[2], Sung Don Lim[2] and Simon Gilroy[1]

Fluorescent protein-based biosensors are providing us with an unprecedented, quantitative view of the dynamic nature of the cellular networks that lie at the heart of plant biology. Such bioreporters can visualize the spatial and temporal kinetics of cellular regulators such as Ca^{2+} and H^+, plant hormones and even allow membrane transport activities to be monitored in real time in living plant cells. The fast pace of their development is making these tools increasingly sensitive and easy to use and the rapidly expanding biosensor toolkit offers great potential for new insights into a wide range of plant regulatory processes. We suggest a checklist of controls that should help avoid some of the more cryptic issues with using these bioreporter technologies.

Addresses
[1] Department of Botany, University of Wisconsin, Birge Hall, 430 Lincoln Drive, Madison, WI 53706, USA
[2] Department of Biochemistry and Molecular Biology, 1664 N. Virginia Street, University of Nevada, Reno, NV 89557, USA

Corresponding author: Gilroy, Simon (sgilroy@wisc.edu)

Current Opinion in Plant Biology 2018, **46**:32–38

This review comes from a themed issue on **Cell biology**

Edited by **Ram Dixit** and **Elizabeth Haswell**

https://doi.org/10.1016/j.pbi.2018.07.004

Introduction

Fluorescent biosensors, that is, fluorescent molecules introduced into an organism to monitor some parameter of its biological activity, have been used for decades to quantify the real-time spatiotemporal dynamics of myriad signaling processes in biology. Although reporter dyes (small organic molecule-based sensors) have been foundational in developing this area [1], the advent of fluorescent protein (FP) technologies, and the associated possibility of engineering genetically encoded indicators, has led to an explosion of research using this approach. Most of these probes have been developed for biomedical applications and then subsequently applied to plant systems. However, we are now beginning to see biosensors designed to report on plant-specific features such as phytohormones [2••,3••,4,5••,6] or transceptor activity [7,8,9•,10], offering new and unique insights into the remarkably dynamic cellular life of the plant.

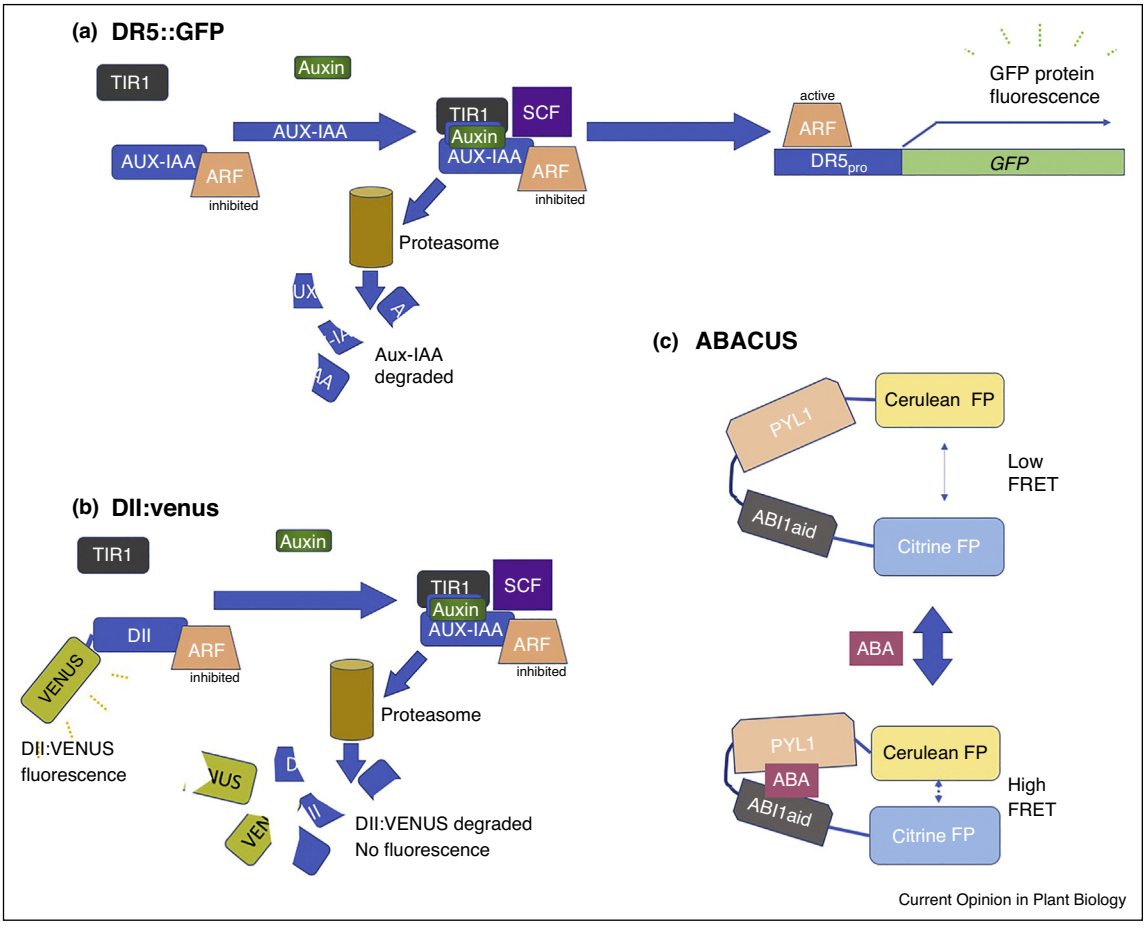
The field of FP-based reporters of biological processes is now immense and so we will limit our discussion to a subset of these technologies. Thus, there are approaches to look at plant hormone signaling responses using *promoter::Green Fluorescent Protein (GFP)* expressing plants (e.g. auxin using *DR5rev::GFP* [11] or cytokinin with *TCSn::GFP* [12]) or protein:FPs that are degraded upon hormone perception (e.g., *DII-VENUS* for auxin [13], *Jas9-VENUS* for jasmonate [14]; Figure 1). These are important tools to explore plant cell function, and for some plant regulators such as auxin and jasmonic acid, remain essentially the only available approaches to follow their cellular dynamics. However, these methodologies monitor the output of the cellular response system triggered by the molecule of interest, for example, auxin regulated gene expression is used to infer auxin dynamics (Figure 1). In this article, we focus on a different family of FP-based sensors where the fluorescence signal is reversibly altered by either direct interaction with the molecule being investigated or in response to a physical parameter of the cell such as membrane voltage (Figure 1c). These sensors allow real-time imaging of the dynamics (both rise and fall kinetics) of cellular components down to the subcellular level.

Genetically-encoded biosensors in plants

The use of genetically-encoded fluorescent biosensors in plants has experienced a renaissance in recent years, owing to the prevalence of sensors developed for animal studies that have been successfully co-opted for plant biology research (Table 1). Generally, sensor design takes an endogenous protein or protein domain sensitive to the parameter of interest and then fuses it to one or more fluorescent proteins. The fusion is made in such a way that the fluorescence of the FP is altered by the conformational change in the sensor domain upon binding of that domain to its cellular ligand or structural rearrangement of the sensor region in response to change in a cellular parameter such as alterations in membrane potential (Figure 1).

Several approaches have been developed to translate this conformational change in the sensor domain to altered

Figure 1



FP-based sensors for plant hormone dynamics. **(a)** With *DR5::GFP*, auxin activates its endogenous signaling network. Normally the auxin response factor/transcriptional activator (ARF) is repressed by its association with an AUX-IAA protein. When auxin binds to its receptor (a TIR/AFB family member protein) a complex forms between the receptor, AUX-IAA and the SCF complex that targets AUX-IAA for degradation in the proteasome [30]. This loss of the AUX-IAA protein relieves ARF repression and allows induction of auxin responsive promoters such as the synthetic *DR5* promoter element. The *DR5* element can then drive GFP production and so GFP fluorescence represents a proxy for auxin activation of the system. **(b)** *DII:VENUS* uses the same signaling cascade but substitutes a synthetic AUX-IAA fused with the VENUS FP variant. The DII:VENUS protein is constitutively expressed but is degraded as auxin levels rise. Loss of fluorescence is used as a measure of auxin activity. DII:VENUS response times are in the minutes time-frame making it more temporally responsive than a transcriptional activator-based reporters such as *DR5::GFP*. **(c)** In the ABACUS bioreporter, The ABA receptor PYL1 forms a protein hinge with a fragment of the ABI1 phosphatase (ABI1aid). Upon ABA binding, the receptor domain binds to the ABI1 fragment causing a conformational change in the biosensor. This change alters the interaction of the two attached fluorescent proteins such that the FRET energy transfer between them is enhanced. Monitoring FRET signal therefore provides a direct, real-time measure of ABA levels. *Abbreviation*: FP, fluorescent protein.

fluorescence of the attached FP. For biosensors based on Förster resonance energy transfer (FRET) the sensory domain governs the relative distance between and/or the relative orientations of two linked FPs, a donor and acceptor [15]. The FPs are carefully chosen such that upon excitation of the donor, the energy it would normally emit as fluorescence is transferred to the acceptor by the process of resonance energy transfer, leading to fluorescence emission from the acceptor. The efficiency of this FRET is proportional to the 6th power of the distance between the fluorophores and is also dependent on the relative orientations of the FPs, making FRET highly sensitive to changes in the positions of the FPs governed by the sensor domain's conformation. In practice 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 artifacts and for differences in expression level and reporter localization [15]. Table 1 lists some examples of FRET-based sensors such as the yellow cameleon family of Ca^{2+} sensors (e.g., YC3.6, YCNano and D4) and hormone sensors (ABACUS, ABAleon and GPS1) that have been successfully used in plants.