Editorial

Seeing is understanding

Check for updates

A plethora of methods is available to study plants, from the humble northern or western blots to recent high-throughput single-cell and spatial transcriptomics. But seeing is believing, and researchers worldwide have always had a weakness for specific genetically encoded biosensors that can isolate and visualize one precise response in living plants at cellular resolution.

Biological organisms are incredibly complex systems that host a phenomenal number of simultaneous biochemical reactions. Local concentrations of metabolites vary constantly; enzymes repeatedly meet their substrates; vesicles are formed and recycled to exchange chemicals and proteins between subcellular compartments; and gene regulatory networks control the production of new proteins that interact with other components and are subsequently destroyed by the proteasome in endless cycles that are stopped only by death. Indeed, this extreme state of dynamic balances is needed for sustaining life itself, and that includes responding quickly to endogenous or external signals by temporarily altering the equilibrium in one direction or another.

The job of molecular biologists is to understand this mess, and it is not a simple task. We must apply some type of reductionist approach and presuppose that one low-level pathway or one limited gene network can be understood in isolation, supposing that the context in which these isolated processes happen stays stable. In other words, we analyse one limited aspect of the system to add knowledge about the whole organism, and assume that the whole can be understood as the sum of its parts. This is of course not just a conceptual choice, as we are constrained by what current methods allow us to measure at a single time point or monitor continuously in live cells.

Fortunately, techniques improve continuously. Molecular biologists used to crush one seedling or a full leaf − destroying all spatial information in the process − to be able to have enough material to know the global level of one transcript, one protein or maybe one metabolite. Classical methods such as western blots or even individual-transcript in situ hybridization and protein immunolabelling are slowly being replaced by heavily parallel approaches that can combine various advantages, such as high-throughput sequencing, 2D or 3D spatial information, multiple and even genome-wide readings at once, non-destructive monitoring, subcellular resolution and so on.

During the first half of 2023 alone, we have published a few principally methodological studies that introduce new approaches in plants, including a powerful combination of single-cell and spatial transcriptomes[1] in soybean to establish a genome-wide cell expression atlas in a histological slice of a symbiotic nodule. In this issue, two papers describe simultaneous transcript and protein quantification and multiplexed hybridization followed by amplification to visualize the expression levels of dozens of genes simultaneously. All of these techniques are applied to fixed tissues, so − despite their obvious resolving power − they cannot be used on live material.

Although these high-throughput techniques are effective, they are not adapted for specific, single-response monitoring. For this type of focused and reductionist approach, genetically encoded biosensors are more adaptable. The first and easiest level to probe gene expression is a simple transcriptional fusion between a promoter and a visible reporter. The reporter is often a fluorescent protein or an enzyme that needs to be processed (GUS or LUC), but can also be visible instantly without any special equipment. This has become possible with developments such as RUBY red betalain production[2] and autoluminescence[3]. A bit more complex is a combination of reporters − for example, we have published on a cell cycle sensor[4] that uses three degradable translational fusions with fluorescent proteins to label nuclei with a specific colour for each cell cycle phase.

Sometimes the focus is not on protein or gene expression levels, but on small molecules such as hormones, ions and metabolites, or even biochemical activity such as phosphorylation. Fluorescent proteins can be engineered into biosensors to transform a chemical property into a quantifiable signal.

Efficient calcium sensors have been built by fusing a single GFP with calmodulin and calmodulin-interacting domains; the binding of Ca^{2+} ions induces a conformational change that markedly increases brightness. In our pages, such a sensor (known as GCaMP6f) was used to visualize calcium dynamics during trap closure[5] in the carnivorous Venus flytrap. An older study[6] used two types of biosensor: engineered YFP fused to cationic tails to label membranes depending on their electrostatic signatures, and YFP fused to specific lipid-binding domains to visualize membrane phosphoinositides. More common is bimolecular fluorescence complementation − another type of fluorescent protein engineering, best described as 'split-YFP' − to monitor protein–protein interactions in vivo.

In some cases, molecular constraints make it easier to use two fluorescent proteins to visualize changes. They are commonly separated by a molecular switch that will bring them closer after the binding of a molecule and inducing Förster resonance energy transfer (FRET), whose ratio can be quantified with a confocal microscope. These types of biosensors seem to work better for small molecules such as hormones. In this month's issue, a study presents a next-generation ABACUS sensor, which is based on two fluorescent proteins separated by domains from abscisic acid (ABA) receptor and coreceptor. The presence of ABA acts as a molecular glue to increase intramolecular interaction, which brings the fluorescent proteins closer together. Various steps of rigorous optimization provided a high affinity for endogenous concentrations of ABA that enables precise mapping of ABA at cellular resolution in an entire seedling. A few years ago, the same laboratory at the University of Cambridge designed a gibberellin FRET biosensor known as GPS1 (ref. 7), again based on the receptor machinery, that could visualize gradients of the hormone in vivo.

The road to an ideal FRET biosensor is empirical and paved with protein engineering pitfalls. We should be thankful to researchers who spend an inordinate amount of time designing these tools and share them with the community. Structural considerations such as binding site accessibility must be taken in account, and structure-guided modifying or stabilizing mutations must be

Editorial

exhaustively tested (for example, to increase the signal-to-noise ratio or detectable concentration ranges). The length and flexibility of linkers used to attach the various domains and fluorescent proteins have a considerable and unpredictable effect. The whole construct must be expressed ubiquitously, and often the addition of a nuclear localization peptide makes the signal sharper. The induced conformational change must be reversible and fast, which is not always the case. Without these characteristics, no real-time dynamics can be observed. Finally (and far from least important), the biosensor must be orthogonal — which simply means that it should not affect any endogenous pathway in any way. This is not trivial, considering that some small molecules are present at minute concentrations in cells and that an overexpressed biosensor could sequester them and modify sensitivity. Alternatively, the receptor domains used in the switch might interfere with endogenous proteins from the same pathway.

These difficulties are nicely illustrated by the painful design of a relatively efficient and direct FRET biosensor for auxin[8], recently published in *Nature*. Over 2,800 variants were designed and tested before a tryptophan sensor was successfully transformed into an auxin sensor. For decades, auxin researchers have had to rely on indirect transcriptional reporters linked with the synthetic promoter DR5 (ref. [9]), then on the rapid auxin-induced degradation of an Aux/IAA domain with the DII-Venus[10] reporter, followed by a ratiometric version of the same that contains a non-degradable internal control, known as R2D2 (ref. [11]). These are still useful reporters for gathering valuable data, but they provide only an indirect estimation of the local auxin concentration.

Biosensors are conceptually simple tools that consist of an input switch or promoter and a visible and quantifiable output, but they are difficult to design and optimize. Fortunately, their efficiency and sensitivity are still being improved and the number of molecules that can be visualized is increasing. The versatility of these highly diverse tools makes them ideal for many applications, from genetic screens to synthetic biology — and, of course, to probe the complex molecular dynamics that occur in a living organism. Each new biosensor opens a narrow window into the inside of cells and illuminates one specific aspect of the whole. To paraphrase what oceanographer Jacques-Yves Cousteau once said, what is a scientist after all, if not a curious person looking through a keyhole, trying to know what's going on.

Published online: 20 July 2023

References

1. Liu, Z. et al. *Nat. Plants* **9**, 515–524 (2023).
2. He, Y., Zhang, T., Sun, H., Zhan, H. & Zhao, Y. *Hortic. Res.* **7**, 152 (2020).
3. Khakhar, A. et al. *eLife* **9**, e52786 (2020).
4. Desvoyes, B., Arana-Echarri, A., Barea, M. D. & Gutierrez, C. *Nat. Plants* **6**, 1330–1334 (2020).
5. Suda, H. et al. *Nat. Plants* **6**, 1219–1224 (2020).
6. Simon, M. L. A. et al. *Nat. Plants* **2**, 16089 (2016).
7. Rizza, A., Walia, A., Lanquar, V., Frommer, W. B. & Jones, A. M. *Nat. Plants* **3**, 803–813 (2017).
8. Herud-Sikimić, O. et al. *Nature* **592**, 768–772 (2021).
9. Ulmasov, T., Murfett, J., Hagen, G. & Guilfoyle, T. J. *Plant Cell* **9**, 1963–1971 (1997).
10. Brunoud, G. et al. *Nature* **482**, 103–106 (2012).
11. Liao, C.-Y. et al. *Nat. Methods* **12**, 207–210 (2015).