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The biosensor toolbox for plant developmental biology

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Plant development is highly interconnected with the metabolic state of tissues and cells. Current research efforts focus on the identification of the links and mechanisms that govern the interplay between metabolic and gene-regulatory networks. Genetically encoded sensors that allow detection of small molecules *in vivo* and at high spatio-temporal resolution promise to be the tools of choice for quantifying and visualizing the dynamics of metabolite flux in plants. We provide an overview about current approaches to measure signaling molecules, such as hormones, calcium and sugars, as well as for monitoring the metabolic state via energy equivalents and pH. Biosensors show great potential to address questions of plant development but there are also limitations where alternative approaches are needed.

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Introduction

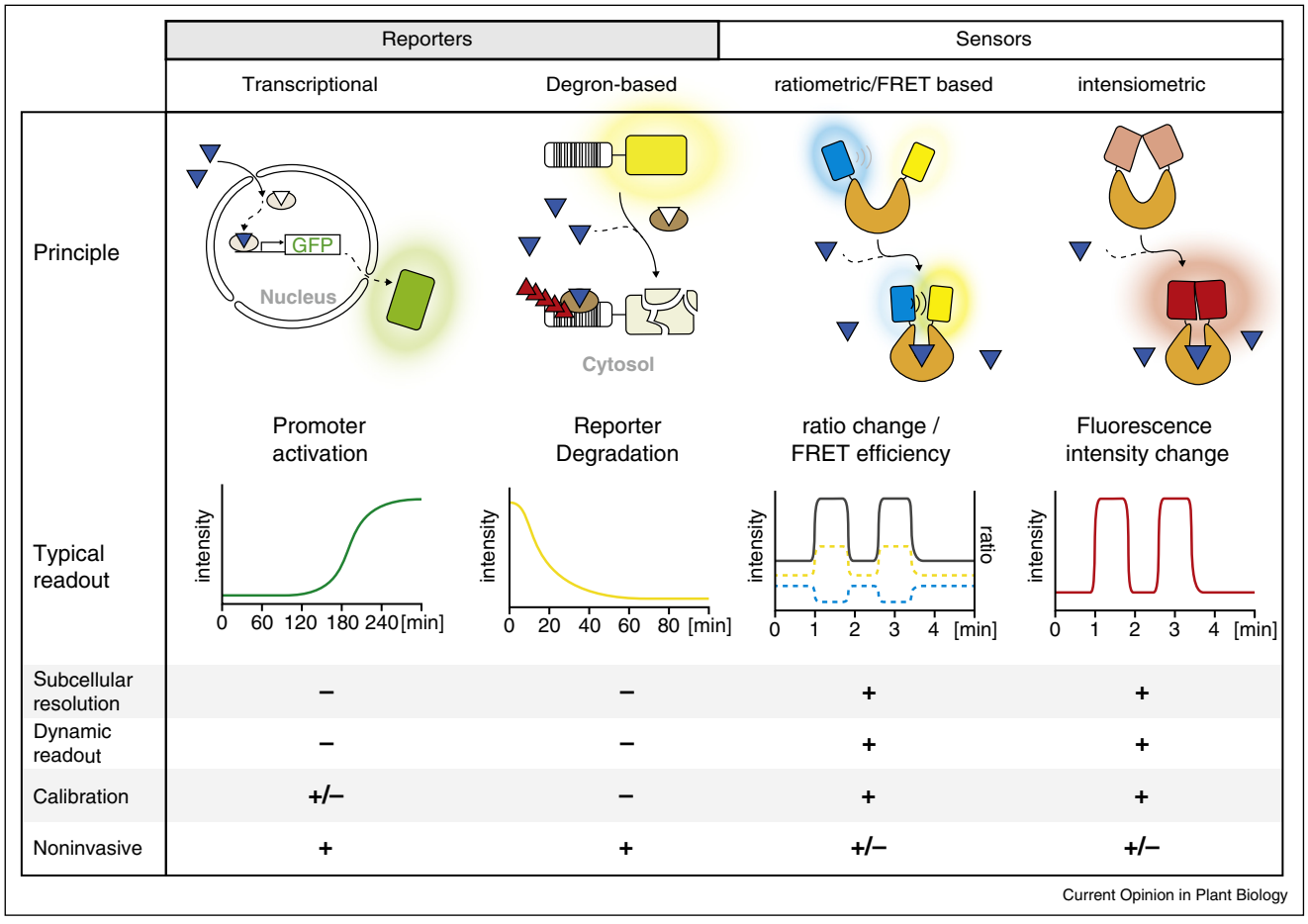
Growing genetically identical plants under different environmental conditions results in different plant morphologies. This widely known fact highlights the ability of plants for plastic adaptation of the body plan to meet the requirements of a sessile lifestyle. Anatomical adaptations can be observed on a wide range of scales such as overall plant size, shoot and root system architecture (RSA), leaf size and lateral root density, down to cellular responses such as the development of trichomes and root hairs. These observations imply that the gene-regulatory pathways are closely interlinked with the physiological state of individual cells and tissues. In particular the hidden part of plants, their root system, has gained increasing attention to study developmental responses to environmental parameters since efficient exploration of the soil by roots is critical for plant nutrition and, hence, for crop yield. In his famous work, John E. Weaver described how soil conditions in the field influenced RSA of crop plants [1]. Ever since, plant scientists have been striving to unveil the links between environmental conditions, physiological state of the plant and postembryonic plant development. Comprehensive analyses of RSA in response to nutrient deficiencies have been performed in crop plants [2] as well as in model plants such as Arabidopsis [3]. Their findings led to the hypothesis that plastic adaptation to the availability of nutrients, water or oxygen, requires systemic signaling of the nutritional, metabolic or energy state. In the case of nitrogen supply, such a systemic signaling has recently been demonstrated [4]. In the case of water, plants are known to adjust their root development to maximize water retention and water uptake efficiency, which is achieved by the asymmetric emergence of root hairs (towards low water potential) or lateral roots (towards higher potential) [5]. In soils of poor phosphorus availability, many plant roots are also able to respond locally and maximize their surface area through adaptations such as formation of cluster roots [6].

To understand how physiology and development are linked, we need methods that directly measure nutrients, metabolites, and signaling molecules in plants *in vivo*. Technological advances over the past decades have made comprehensive analyses of physiological state of plants possible. While mass spectrometry-based approaches allow for metabolic profiling of plants, several minimally invasive techniques have been developed that allow for the measurement of metabolites *in vivo* and are therefore suited to assess metabolic dynamics in living plants. In this review we are focusing on the application of genetically encoded fluorescent sensors and reporters that directly provide information on changing levels of small molecules with high spatiotemporal resolution. To distinguish between sensors and reporters we adopt a previously established definition [7]; firstly, sensors bind the detected molecule directly and reversibly, allowing the detection of dynamic changes in substrate concentration; secondly, reporters respond indirectly by depending on a cell-endogenous detection system, thus typically lacking reversibility but featuring higher sensitivity (Figure 1).

Calcium sensors

Plant development depends on intercellular communication, which involves various messenger molecules such as calcium, reactive oxygen species (ROS), hormones, peptides or small RNAs [8,9]. Among these, calcium is the most ubiquitous and versatile messenger molecule with

Figure 1

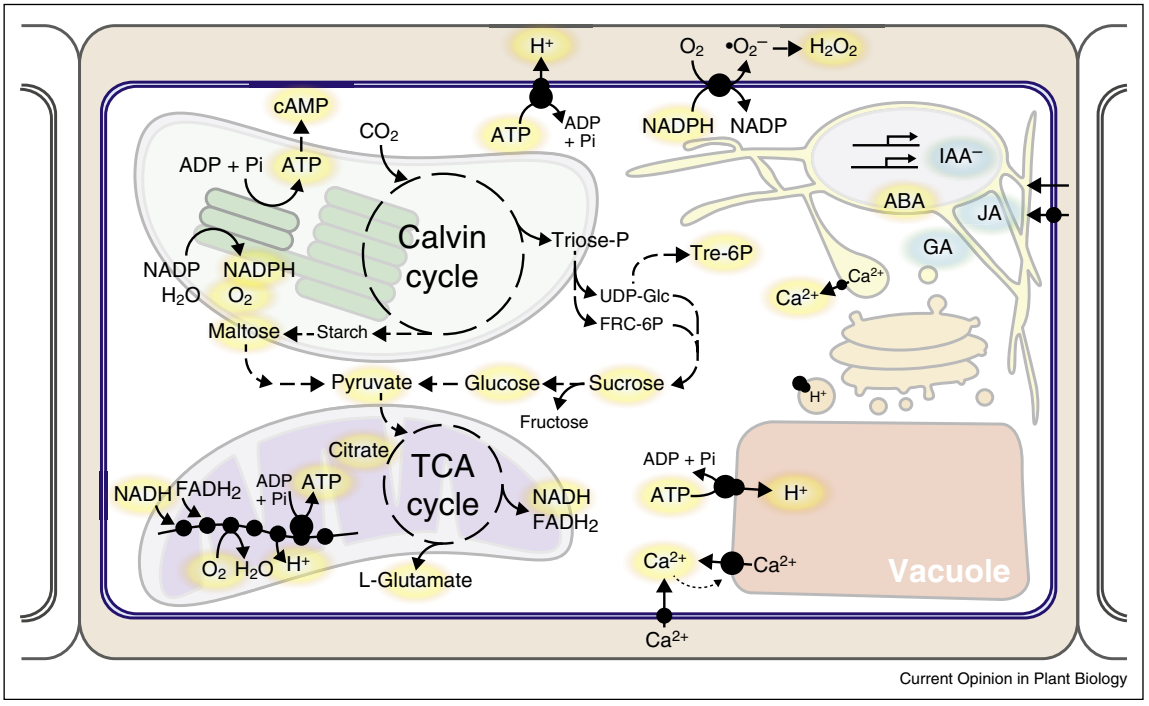


Genetically encoded reporters and biosensors for the detection of small molecules *in vivo*. Based on their working principle, both reporters and sensors are divided into two subclasses each: transcriptional reporters and degron-based reporters; FRET based sensors and single fluorophore sensors. Each of these approaches has specific advantages and limitations regarding the temporal resolution, their effect of endogenous signaling (invasiveness), and the correlation between the amount of detected target molecule and readout (calibration). Blue triangles depict target molecules. Red triangles depict ubiquitin molecules. Fluorescent proteins are depicted as rounded rectangles, glow illustrates their relative emission intensity.

known functions that range from signal perception in the cell periphery to transcriptional regulation in the nucleus (Figure 2). Biotic and abiotic stresses as well as the perception of mechanical force are linked to developmental responses via transient elevations of cytosolic calcium levels [9–11]. Information on the stimulus is encoded in the specific spatio-temporal patterns (signatures) that are then decoded by a complex network of calmodulin, calmodulin-like proteins, calcium dependent protein kinases, Calcineurin-B-like proteins (CBLs), and CBL-interacting protein kinases [12], eventually resulting in signature-specific gene expression [13].

To reveal amplitudes and spatiotemporal information of calcium signatures it is necessary to visualize cytosolic calcium levels ($[Ca^{2+}]_{cyto}$) *in vivo*. Due to its ubiquitous importance throughout all kingdoms of life, calcium was one of the first target molecules for *in vivo* measurements. Over the past decades, sensitive dyes (e.g. FURA), calcium responsive luminescent proteins (e.g. Aequorin) and fluorescence-based calcium sensors have been developed. A distinction is that calcium sensors can be genetically encoded and do not require the addition of luminogenic substrates. A major breakthrough in the history of genetically encoded calcium indicators (GECIs) was the development of 'cameleon' sensors [14] and their immediate adoption for live imaging of cytosolic calcium levels in plant cells [15]. The design principle of these sensors has served as a template for many sensors to come; a substrate-specific binding domain (here: a fusion of calmodulin and the calmodulin-binding peptide M13) is fused to two spectrally overlapping fluorophores, able to exert non-radiative Förster Resonance Energy Transfer (FRET). Any conformational changes in the binding domain upon

Figure 2



In vivo measurements of metabolites in plant cells. Metabolic and signaling pathways can be directly addressed using genetically encoded fluorescent sensors. Highlighted molecules indicate the availability of specific sensors; yellow: rapid direct readout, for example via FRET-based sensors; blue: degron-based sensors.

substrate binding are translated into a change in distance and/or orientation of the two fluorophores to each other and thereby alter FRET efficiency (Figure 1). Thanks to its large dynamic range (560%) and brightness the variant yellow cameleon 3.60 (YC3.60) [16] has become the most widely used cameleon sensor in plant studies [17–24,25•]. YC variants with substantially improved Ca^{2+} affinity (K_d = 15–140 nM) and dynamic range (1450%) have been developed (YC-Nano; [26]) and recently also utilized to reveal long-distance communication in Arabidopsis upon localized exposure to salt stress [27••]. To avoid potential interference with endogenous calmodulin signaling, the binding domain of myocyte-specific troponin C (TnC) has been combined with brighter and more photostable fluorophores to yield GECIs of the TN-L and Twitch families [28,29]. For example, cell type-specific expression of CerTN-L15 has been used to disclose specific calcium signatures in synergids, egg and central cells inside Arabidopsis ovules during double fertilization, where continuous recording over several hours was required [30].

Another major advantage of fluorescence-based sensors over other forms of detecting $[Ca^{2+}]_{cyto}$ changes is the possibility to achieve cell-type specific expression or even target them to subcellular compartments [21]. For example, fusion of sensors to a nuclear localization sequence facilitates the tracking of responses in individual cells, because nuclei can be clearly optically separated in most plant tissues.

In recent years, the palette of GECIs has been extended by the development of single fluorophore sensors, which translate the ligand-induced conformational change in the binding domain into a structural stretching of circular permuted variants of fluorescent proteins (cpFP) and thus a change in fluorescence intensity (Figure 1). GCaMPs and the more recently developed GECOs [31,32] feature high dynamic range (up to 1600% intensity increase) and are available in various emission spectra from blue to red. In a comparative study, the red-fluorescent R-GECO was able to report even subtle oscillations in the $[Ca^{2+}]_{cyto}$ signatures, where YC3.60 also detected $[Ca^{2+}]_{cyto}$ transients but was unable to resolve any sub-features [33•].

Sugar sensors

The development of the first fluorescent sensors for sugars marked the beginning of *in vivo* imaging of metabolites [34,35]. It was demonstrated that the design principle of calcium FRET sensors — a substrate-binding domain linked to two fluorophores — could be adapted to generate sensors also for larger molecules. Assuming that specific binding proteins exist for most nutrients, metabolites and signaling molecules, the possibilities for sensor design seem limitless (Figure 2).

Meanwhile, FRET-based sensors for glucose, galactose [36–39], maltose, arabinose [40], ribose [41] and sucrose [42] as well as for the important sugar signaling molecule trehalose-6-phosphate (T6P) [43] exist and the palette is still growing. Importantly, to allow measurements in diverse cell types and cellular compartments, most sugar sensors have been developed as different affinity variants, with K_d values ranging from nM to mM substrate concentrations. Most applications for sugar sensors have so far focused on measuring transport kinetics [44,45] and have, for example, lead to the identification of a novel sugar transporter family (SWEETs [46]). Sugars are, however, also of particular interest for developmental biology. It is long known that the availability of sugars controls meristem activity [47]; but how photoassimilate abundance in meristems is involved in transcriptome reprogramming via the Glucose-TOR (target of rapamycin) pathway was only recently uncovered [48]. A recent publication reported the surprising finding that after decapitation of the plant shoot, carbon reallocation drives axillary bud release rather than a long suspected activation by the phytohormone auxin [49]. Many more examples exist that demonstrate the pivotal role of sugars in plant development[50]. For example, levels of the signaling-sugar T6P are rising in metabolically active cells and stimulate growth by inhibiting sucrose non-fermenting related kinase 1 (SnRK1), a repressor of protein translation [51]. The recently developed T6P sensor has so far only been applied in bacteria and yeast [43] but is a promising tool for studies on metabolic signaling in plants. In general, measuring sugar flux *in vivo* using genetically encoded sugar sensors has great potential to reveal the molecular interplay between sugar dynamics, cellular signaling and gene regulatory networks and will aid discovery of mechanisms of growth regulation and patterning.

Sensors for energy metabolism

Energy metabolism is not only required for the maintenance of cellular homeostasis but also for the differentiation of cells [52,53]. For example, glycolysis governs the ATP production in the human embryonic stem cells, whereas the tricarboxylic acid (TCA) cycle is the dominant ATP source in differentiated cells [54]. Intriguingly, it has been shown in mammals that Sirtuin1, which influences stem cell fate, is activated when NAD^+ levels exceed those of NADH [55,56]. In plants NAD and its derivatives have been implicated in pollen development and seed production beyond their critical roles in mitochondrial energy metabolism [57]. A recently developed fluorescent biosensor (SoNar) has been used to investigate the NAD^+/NADH ratio in human cell lines to infer their metabolic state [58]. SoNar is based on the NAD^+/NADH binding protein Rex and a circularly permuted yellow fluorescent protein (cpYFP) [59,60]. Depending whether Rex binds NAD^+ or NADH, cpYFP exhibits altered excitation spectra. This spectral shift can be used to determine the NAD^+/NADH ratio. Since lower NAD^+/NADH ratio correlated with proliferative activity in several mammalian cancer cell lines, SoNar was used in a screen for anti-cancer drugs [58].

In addition to NAD^+/NADH, cytosolic ATP itself is the main energy provider for a vast number of cellular activities and protein modifications, important for development and growth of plants (Figure 2). Imamura *et al.* developed a FRET sensor, called ATeams, to measure cytosolic or organelle specific ATP levels [61]. This FRET sensor is based on ATP binding-PS3 ε subunit of bacterial F_0F_1-ATP synthase. When PS3 ε is bound to ATP, FRET efficiency between CFP and mVenus increases. GO-ATeam, which is a further developed version of ATeam sensor has been used to show that, at the single cell level, calcium signaling increases ATP consumption, which further triggers ATP synthesis in mitochondria in a calcium dependent manner [62].

Not only the ATP concentration but also the ratio of cytosolic ATP to ADP is a critical parameter of cellular energy state and can drive several signaling cascades including modulation of AMPK activity in mammalian cells [63]. In Arabidopsis thaliana, SnRK1, the orthologue of AMPK, modulates plant growth particularly in the dark [64]. The sensor for monitoring the molecular dynamics and kinetics of ATP/ADP equilibrium has been named PercevalHR [65], which uses a working mechanism similar to that of SoNar. PercevalHR is composed of an ATP/ADP binding GlnK protein and circularly permutated mVenus fluorescent protein. The fluorescence excitation wavelength changes with respect to the binding partner of GlnK and reflect ATP/ADP ratio [66]. The use of PercevalHR has so far been applied to only mammalian systems to shed light on the link between ATP/ADP ratio and extracellular glucose or K_{ATP} Channel activity [65].

Sensors for reactive oxygen species

Besides the importance of cytosolic ATP, extracellular ATP is a major signaling molecule in regulation of various immune responses, root hair growth, and gravitropism [67]. ATP release to extracellular space leads to production of reactive oxygen species (ROS) by transferring electrons from NADPH and impedes, for example, pollen-tube growth in a calcium and nitric oxide dependent manner [67,68]. ROS together with calcium, pH and cellular redox status are postulated to be crucial signals in modulation of cell-to-cell communication and systemic responses under stress conditions [69,70]. Moreover, ROS has a critical role in loosening the cell wall and promoting cell growth in plant organogenesis [71] (Figure 2). In order to detect ROS *in vivo*, two approaches are commonly used: the single cpYFP-based mitoflash probe [72] (its specificity has recently been questioned, see section 'Limitations of biosensors') and HyPer, a single fluorescent ROS sensor [73]. HyPer directly reports H_2O_2 levels and is therefore to be distinguished from sensors

responding to redox levels (roGFPs, reviewed in [70]). Combined use of HyPer and a calcium sensor *in planta* revealed a calcium-dependent stimulation of hydrogen peroxide catabolism in peroxisomes [74]. A red-fluorescent variant of HyPer was critical in the elucidation of transient peroxide production upon calcium uptake from ER in cultured mammalian cells [75•]. Intriguingly, in plants, it has been shown that low oxygen levels may decrease ATP concentration and induction of sucrose degradation, which is later on observed as a morphological phenotype [76]. In order to detect the molecular oxygen, a FRET-based FluBO bio-sensor was designed in *E. coli*. It is based on the FRET between an oxygen sensitive YFP chromophore and the hypoxia-tolerant flavin-binding fluorescent protein [77].

pH sensors

One of the main determinants of the dynamics and kinetics of metabolic reactions, as for any chemical reaction, is the pH of the solution in which the reaction takes place. Protons also constitute the driving force for numerous transport processes across endomembranes and the plasma membrane, thus being central for ion exchange, nutrition and signaling [78,79] (Figure 2). Moreover, plant growth and stress response require rapid and substantial amount of ion transport for fluid balance and further metabolic reactions, which is regulated by the pH difference of subcellular compartments. For example, stomata closure in the presence of ABA takes place via alteration of pH in the guard cell vacuoles [80]. The regulation of pH homeostasis and generation of proton during plant development has therefore attracted much attention [79,81,82]. pH sensitive dyes such as the green fluorescent probe 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF) have been very useful, for example in revealing that vacuole biogenesis occurs via acidification of ER-derived provacuoles, thus omitting ER-Golgi and post-Golgi trafficking [83]. The demand for measuring pH in living cells has, over the past two decades, driven the development of several biosensors. Many of the available pH sensors (reviewed in [84]) are taking advantage of the pH-sensitivity of GFP, which results in altered excitation and emission spectra. While all biosensors are generally well suited to provide information about qualitative changes in substrate levels, methods for careful calibration of pH sensors have been developed making them valuable tools to obtain quantitative data and determine absolute concentrations. An overview over the distribution of intracellular pH was obtained by changing the sensitivity range of pH biosensors and directing them to different cellular compartments [85]. Furthermore alternative FRET based pH biosensors have become available, for example a CFP-YFP tandem protein, in which the FRET efficiency is lost in acidic conditions. This biosensor has been used to show the influence of pain sensing TRPV1 channels in cytosolic pH in nociceptive neurons [86]. Using the ratiometric sensor pHusion a recent study demonstrated the link between luminal pH of the trans-golgi network/early endosome (TGN/EE) and exocytosis and recycling [87••]. A mutated TGN V-ATPase or treatment with concanamycin A resulted in increased luminal pH and affected TGN/EE motility and secretion. An apolast-targeted version of pHusion (apo-pHusion) has been employed to show that external auxin application causes rapid alkalinization of the subepidermal apoplast [88].

Hormonal signaling — sensors and reporters

Developmental plasticity depends on communication and coordination mediated by a complex network of phytohormones, which are critical for growth and development as well as for systemic responses to alterations of environmental conditions and metabolic state [89]. In order to visualize the local activity of auxin (IAA) at the cellular level a transcriptional reporter based on the auxin inducible promoter DR5 was developed [90] (Figure 1). This reporter has served as IAA response indicator in hundreds of studies. The transcriptional reporter becomes activated about 2 hours after the auxin signaling begins, which limits the analysis of the timing of auxin redistributions, for example upon gravistimulation of the root. Since DR5 can only be activated in cells that are competent for perceiving auxin, response intensity depends on cell-type and developmental stage.

To substantially increase the temporal resolution of visualizing auxin signaling the DII degron sequence of Aux/IAA repressor proteins was fused to the YFP variant VENUS including a nuclear localization signal. Elevated intracellular auxin levels therefore lead to a drop of fluorescence of the DII-Venus reporter within tens of minutes [91] (Figure 1). This allowed visualizing asymmetric auxin levels within 30 min of gravistimulation, indicated by a decrease of fluorescence signal on the lower side of the root (towards the gravity vector), whereas the signal was maintained in tissues of the upper side. The combination of DII-Venus and a mutated red-fluorescent mDII-Tomato resulted in the development of a ratiometric, semiquantitative auxin reporter named R2D2 that facilitates monitoring auxin signaling at high spatiotemporal resolution [92••].

Using a similar degron-based design, a reporter for gibberellic acid (GA) has been developed by utilizing degradation of the DELLA protein REPRESSOR OF GA1-3 (RGA) downstream of GA signaling [93]. It was known that auxins and GA signaling are linked during gravity response and attempts to directly measure the relocalization of both hormones had been undertaken in the past [94,95]. With the new GFP-RGA reporter, researchers managed to pin down the temporal dynamics of GA levels within 3 hours after gravitational stimulus [93].

Auxin signaling is also tightly interconnected with other signaling pathways such as jasmonic acid (JA) signaling [96]. For instance, JA represses PLETHORA genes, which are critical for auxin signaling [97]. On the other hand, auxins upregulate several JAZ proteins, which subsequently suppress JA signaling [89]. JA signaling negatively regulates root apical meristem size by antagonizing auxin signaling. In order to monitor JA signaling, a Jas9-VENUS fluorescent reporter has been developed [98•]. Jas9-VENUS is the third available degron-based reporter as it makes use of the Jas domain of Jasmonate ZIM (JAZ) domain proteins, which are targeted to SCF^{COI1} E3 ubiquitin ligase in the presence of active JA signaling. As a result, the VENUS signal decreases where JA signaling is active. With this reporter, a high-resolution spatiotemporal map of JA signaling was generated and the dynamics of long-distance JA signaling were addressed upon wounding [98•].

Abscisic acid (ABA) is another central player in the tightly interconnected metabolic signaling in plants and is involved in the acclimation of plants to environmental conditions such as water shortage or colder temperatures [99,100]. ABA has been further implicated in modulation of seedling growth, embryo maturation, floral induction, and leaf patterning during plant development. The developmental role of ABA was revealed by external ABA application or mutant plant lines, which either cannot produce ABA or are insensitive to ABA. Therefore, the spatiotemporal dynamics of endogenous ABA remained elusive [101,102].

Recently, a major milestone was achieved with the development of the first directly measuring hormone sensors. Two independent studies succeeded in establishing FRET-based biosensors for ABA, ABAleon [103••] and ABACUS [104••]. Both sensors are FRET-based and are using the ABA-induced interaction of a receptor of the PYR/PYL/RCAR family with the physically linked co-receptor ABI1 of the protein phosphatase 2CA (PP2CA) family. ABAleon responds with a reduced intensity ratio of FRET-donor and acceptor upon ABA binding, while the FRET ratio increases in ABACUS in the presence of ABA. The initial response of both sensors is detectable within a few seconds after ABA administration. Combined with confocal microscopy, Waadt *et al.* and Jones *et al.* both measured the kinetics of ABA transport and cytosolic ABA dynamics in an organ, tissue and cell-type-specific manner. While the sensors proved very responsive to externally applied ABA, measuring endogenous changes of ABA levels, for example during salt stress turned out to be more difficult [104••]. This highlights the special needs in sensitivity when developing sensors for phytohormones. Nonetheless, the developments of both ABA sensors have demonstrated the potential of biosensors for elucidating the intracellular and intercellular dynamics of phytohormones *in vivo*.

Limitations of biosensors

Despite the advantages of biosensors over the use of reporters, there are also limitations where the use of sensors may result in undesirable side effects or the generation of artifacts. When employing biosensors as the only means of detecting substrate dynamics it is necessary to determine whether the measured sensor response is indeed caused by a change in substrate concentration or by other factors that influence the readout. Inconclusive results may be obtained due to insufficient substrate specificity of the sensor or its sensitivity to environmental changes such as pH or ion strength. As an example, the usability of circular permuted YFP (cpYFP) for measuring superoxide has been questioned and sensor responses were instead attributed to alterations in pH [105]. When using FRET sensors, it has to be considered that both fluorophores may be affected differently, which almost certainly results in a change in FRET efficiency. Also differential photostability of both fluorophores would influence any time lapse measurement. Many of these issues can be accounted for by including the proper controls. Also the choice of fluorophores can help avoiding undesired cross reactions. For instance, the previously widely used FRET donor eCFP is susceptible to high concentrations of ATP, which was not observed for newer cyan variants such as mCerulean [106].

In the case of ABA sensors, there were indications that the higher the substrate affinity of the sensor or the higher its expression level, the more likely plants became less responsive to ABA, potentially due to sequestration of ABA by the sensor [103••]. This highlights the need to assess whether a directly binding sensor or an indirectly acting reporter are more suitable tools to analyze the dynamics or a particular substrate. The substantially higher spatiotemporal resolution of sensors comes at the cost of lower sensitivity and potentially higher invasiveness (Figure 1). In many cases it may be necessary to combine sensor measurements with reporters or other means of substrate detection.

Future perspectives

Metabolic networks determine the growth and development of plants as well as their reproduction and senescence. Intricate networks of metabolic reactions are regulated by nutrient availability, environmental conditions and gene regulatory networks. Our current knowledge of metabolic reactions is based on their biochemical analysis *in vitro* or by genetic manipulations of the enzymes that drive these reactions. We therefore need genetically encoded biosensors that detect metabolites directly to reveal the kinetics of metabolite flux and the links of metabolic state and development *in vivo*.

We have seen on the example of calcium sensors that continuous improvement of biosensor brightness, sensitivity and affinity can substantially enhance spatial and

temporal resolution, improve reliability of the readout and minimize interference caused by sensor expression. On the other hand, sensors can also be designed to actively take part in an enzymatic reaction, thus reporting on the conformational change during catalytic activity. This notion coined the term '*in vivo* biochemistry' [107], which refers to the analysis of kinetic and structural features of enzymes in their cellular environment.

Although biosensors have been used predominantly for detection of qualitative changes, a rarely explored potential of biosensors is the possibility to obtain quantitative data on substrate concentrations. Lanquar *et al.* used a combination of several affinity variants of the FRET-based eCALWY sensors for Zn^{2+} and pulsed treatments with Zn^{2+} chelators and ionophores to calibrate the sensors in plant cells [108]. The measured subcellular concentrations of Zn^{2+} were matching previous results obtained by destructive techniques.

While we focused in this review on sensors for molecules involved in signaling during plant development, there are numerous biosensors for metabolites and nutrients available of which only a small number has been applied in the plant field. Future studies will harness possibilities to combine measurements of multiple signals simultaneously or the sensing of signaling molecules with dynamic measurements of nutrients such as for nitrogen [109•,110], phosphorus [111,112] or metals [108]. A promising approach represents the development of single fluorophore sensors as they allow the combination of different sensors with little spectral overlap.

Genetically encoded sensors as fluorescent probes for small molecules have substantially extended the toolbox for plant sciences (Figure 2). Through their continued development and improvement, visualizing dynamic processes in living organisms has gained a momentum that promises fascinating new insights how metabolic and gene regulatory networks are linked during the plastic development of plants.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Weaver JE: *Root Development of Field Crops*. 1st ed.. New York: McGraw-Hill; 1926.

2. Hodge A: **The plastic plant: root responses to heterogeneous supplies of nutrients**. *New Phytol* 2004, **162**:9-24.

3. Gruber BD, Giehl RFH, Friedel S, Wirén von N: **Plasticity of the Arabidopsis root system under nutrient deficiencies**. *Plant Physiol* 2013, **163**:161-179.

4. Tabata R, Sumida K, Yoshii T, Ohyama K, Shinohara H, Matsubayashi Y: **Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling**. *Science* 2014, **346**:343-346.

5. Bao Y, Aggarwal P, Robbins NE, Sturrock CJ, Thompson MC, Tan HQ, Tham C, Duan L, Rodríguez PL, Vernoux T *et al.*: **Plant roots use a patterning mechanism to position lateral root branches toward available water**. *Proc Natl Acad Sci USA* 2014, **111**:9319-9324.

6. Lambers H, Martinoia E, Renton M: **Plant adaptations to severely phosphorus-impoverished soils**. *Curr Opin Plant Biol* 2015, **25**:23-31.

7. Lemke EA, Schultz C: **Principles for designing fluorescent sensors and reporters**. *Nat Chem Biol* 2011, **7**:480-483.

8. Van Norman JM, Breakfield NW, Benfey PN: **Intercellular communication during plant development**. *Plant Cell* 2011, **23**:855-864.

9. Kudla J, Batistič O, Hashimoto K: **Calcium signals: the lead currency of plant information processing**. *Plant Cell* 2010, **22**:541-563.

10. Monshausen GB, Bibikova TN, Weisenseel MH, Gilroy S: **Ca^{2+} regulates reactive oxygen species production and pH during mechanosensing in Arabidopsis roots**. *Plant Cell* 2009, **21**:2341-2356.

11. Richter GL, Monshausen GB, Krol A, Gilroy S: **Mechanical stimuli modulate lateral root organogenesis**. *Plant Physiol* 2009, **151**:1855-1866.

12. Hashimoto K, Kudla J: **Calcium decoding mechanisms in plants**. *Biochimie* 2011, **93**:2054-2059.

13. Whalley HJ, Knight MR: **Calcium signatures are decoded by plants to give specific gene responses**. *New Phytol* 2013, **197**:690-693.

14. Miyawaki A, Llopis J, Heim R, McCaffery JM, Adams JA, Ikura M, Tsien RY: **Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin**. *Nature* 1997, **388**:882-887.

15. Allen GJ, Kwak JM, Chu SP, Llopis J, Tsien RY, Harper JF, Schroeder JI: **Cameleon calcium indicator reports cytoplasmic calcium dynamics in Arabidopsis guard cells**. *Plant J* 1999, **19**:735-747.

16. Nagai T, Yamada S, Tominaga T, Ichikawa M, Miyawaki A: **Expanded dynamic range of fluorescent indicators for Ca^{2+} by circularly permuted yellow fluorescent proteins**. *Proc Natl Acad Sci USA* 2004, **101**:10554-10559.

17. Iwano M, Shiba H, Miwa T, Che F-S, Takayama S, Nagai T, Miyawaki A, Isogai A: **Ca^{2+} dynamics in a pollen grain and papilla cell during pollination of Arabidopsis**. *Plant Physiol* 2004, **136**:3562-3571.

18. Monshausen GB, Messerli MA, Gilroy S: **Imaging of the Yellow Cameleon 3.6 indicator reveals that elevations in cytosolic Ca^{2+} follow oscillating increases in growth in root hairs of Arabidopsis**. *Plant Physiol* 2008, **147**:1690-1698.

19. Rincón-Zachary M, Teaster ND, Sparks JA, Valster AH, Motes CM, Blancaflor EB: **Fluorescence resonance energy transfer-sensitized emission of yellow cameleon 3.60 reveals root zone-specific calcium signatures in Arabidopsis in response to aluminum and other trivalent cations**. *Plant Physiol* 2010, **152**:1442-1458.

20. Chabaud M, Genre A, Sieberer BJ, Faccio A, Fournier J, Novero M, Barker DG, Bonfante P: **Arbuscular mycorrhizal hyphopodia and germinated spore exudates trigger Ca^{2+} spiking in the legume and nonlegume root epidermis**. *New Phytol* 2011, **189**:347-355.

21. Krebs M, Held K, Binder A, Hashimoto K, Herder Den G, Parniske M, Kudla J, Schumacher K: **FRET-based genetically encoded sensors allow high-resolution live cell imaging of Ca^{2+} dynamics**. *Plant J* 2012, **69**:181-192.

22. Iwano M, Ngo QA, Entani T, Shiba H, Nagai T, Miyawaki A, Isogai A, Grossniklaus U, Takayama S: **Cytoplasmic Ca^{2+} changes dynamically during the interaction of the pollen tube with synergid cells**. *Development* 2012, **139**:4202-4209.

23. Boisson-Dernier A, Lituiev DS, Nestorova A, Franck CM, Thirugnanarajah S, Grossniklaus U: **ANXUR receptor-like kinases coordinate cell wall integrity with growth at the pollen tube tip via NADPH oxidases**. *PLoS Biol* 2013, **11**:e1001719.

24. Hamamura Y, Nishimaki M, Takeuchi H, Geitmann A, Kurihara D, Higashiyama T: **Live imaging of calcium spikes during double fertilization in Arabidopsis**. *Nat Commun* 2014, **5**:4722.

25. • Behera S, Wang N, Zhang C, Schmitz-Thom I, Strohkamp S, Schultke S, Hashimoto K, Xiong L, Kudla J: **Analyses of Ca^{2+} dynamics using a ubiquitin-10 promoter-driven Yellow Cameleon 3.6 indicator reveal reliable transgene expression and differences in cytoplasmic Ca^{2+} responses in Arabidopsis and rice (*Oryza sativa*) roots**. *New Phytol* 2015, **206**:751-760.
Showing differences in the calcium dynamics in Arabidopsis and rice, this paper highlights the need for comparative studies.

26. Horikawa K, Yamada Y, Matsuda T, Kobayashi K, Hashimoto M, Matsu-ura T, Miyawaki A, Michikawa T, Mikoshiba K, Nagai T: **Spontaneous network activity visualized by ultrasensitive Ca^{2+} indicators, yellow Cameleon-Nano**. *Nat Methods* 2010, **7**:729-732.

27. •• Choi W-G, Toyota M, Kim S-H, Hilleary R, Gilroy S: **Salt stress-induced Ca^{2+} waves are associated with rapid, long-distance root-to-shoot signaling in plants**. *Proc Natl Acad Sci USA* 2014, **111**:6497-6502.
This study demonstrated for the first time that long distance calcium waves are involved in information propagation between root and shoot.

28. Heim N, Garaschuk O, Friedrich MW, Mank M, Milos RI, Kovalchuk Y, Konnerth A, Griesbeck O: **Improved calcium imaging in transgenic mice expressing a troponin C-based biosensor**. *Nat Methods* 2007, **4**:127-129.

29. Thestrup T, Litzlbauer J, Bartholomäus I, Mues M, Russo L, Dana H, Kovalchuk Y, Liang Y, Kalamakis G, Laukat Y *et al.*: **Optimized ratiometric calcium sensors for functional in vivo imaging of neurons and T lymphocytes**. *Nat Methods* 2014, **11**:175-182.

30. Denninger P, Bleckmann A, Lausser A, Vogler F, Ott T, Ehrhardt DW, Frommer WB, Sprunck S, Dresselhaus T, Grossmann G: **Male–female communication triggers calcium signatures during fertilization in Arabidopsis**. *Nat Commun* 2014, **5**:4645.

31. Zhao Y, Araki S, Wu J, Teramoto T, Chang Y-F, Nakano M, Abdelfattah AS, Fujiwara M, Ishihara T, Nagai T *et al.*: **An expanded palette of genetically encoded Ca^{2+} indicators**. *Science* 2011, **333**:1888-1891.

32. Akerboom J, Rivera JDV, Guilbe MMR, Malave ECA, Hernandez HH, Tian L, Hires SA, Marvin JS, Looger LL, Schreiter ER: **Crystal structures of the GCaMP calcium sensor reveal the mechanism of fluorescence signal change and aid rational design**. *J Biol Chem* 2009, **284**:6455-6464.

33. • Keinath NF, Waadt R, Brugman R, Schroeder JI, Grossmann G, Schumacher K, Krebs M: **Live cell imaging with R-GECO1 sheds light on flg22- and chitin-induced transient [Ca^{2+}]cyt patterns in Arabidopsis**. *Mol Plant* 2015, **8**:1188-1200.
Plant lines that co-express two different calcium sensors allowed direct comparison between the ratiometric YC3.6 and the novel intensiometric R-GECO sensor. The higher dynamic range of R-GECO revealed previously unknown features of certain calcium signatures.

34. Fehr M, Frommer WB, Lalonde S: **Visualization of maltose uptake in living yeast cells by fluorescent nanosensors**. *Proc Natl Acad Sci USA* 2002, **99**:9846-9851.

35. Deuschle K, Fehr M, Hilpert M, Lager I, Lalonde S, Looger LL, Okumoto S, Persson J, Schmidt A, Frommer WB: **Genetically encoded sensors for metabolites**. *Cytometry A* 2005, **64**:3-9.

36. Fehr M, Lalonde S, Lager I, Wolff MW, Frommer WB: ***In vivo* imaging of the dynamics of glucose uptake in the cytosol of COS-7 cells by fluorescent nanosensors**. *J Biol Chem* 2003, **278**:19127-19133.

37. Deuschle K, Okumoto S, Fehr M, Looger LL, Kozhukh L, Frommer WB: **Construction and optimization of a family of genetically encoded metabolite sensors by semirational protein engineering**. *Protein Sci* 2005, **14**:2304-2314.

38. Takanaga H, Chaudhuri B, Frommer WB: **GLUT1 and GLUT9 as major contributors to glucose influx in HepG2 cells identified by a high sensitivity intramolecular FRET glucose sensor**. *Biochim Biophys Acta* 2008, **1778**:1091-1099.

39. Veetil JV, Jin S, Ye K: **A glucose sensor protein for continuous glucose monitoring**. *Biosens Bioelectron* 2010, **26**:1650-1655.

40. Kaper T, Lager I, Looger LL, Chermak D, Frommer WB: **Fluorescence resonance energy transfer sensors for quantitative monitoring of pentose and disaccharide accumulation in bacteria**. *Biotechnol Biofuels* 2008, **1**:11.

41. Lager I, Fehr M, Frommer WB, Lalonde S: **Development of a fluorescent nanosensor for ribose**. *FEBS Lett* 2003, **553**:85-89.

42. Lager I, Looger LL, Hilpert M, Lalonde S, Frommer WB: **Conversion of a putative agrobacterium sugar-binding protein into a FRET sensor with high selectivity for sucrose**. *J Biol Chem* 2006, **281**:30875-30883.

43. Peroza EA, Ewald JC, Parakkal G, Skotheim JM, Zamboni N: **A genetically encoded Förster resonance energy transfer sensor for monitoring in vivo trehalose-6-phosphate dynamics**. *Anal Biochem* 2015, **474**:1-7.

44. Chaudhuri B, Hörmann F, Frommer WB: **Dynamic imaging of glucose flux impedance using FRET sensors in wild-type Arabidopsis plants**. *J Exp Bot* 2011, **62**:2411-2417.

45. Grossmann G, Guo W-J, Ehrhardt DW, Frommer WB, Sit RV, Quake SR, Meier M: **The RootChip: an integrated microfluidic chip for plant science**. *Plant Cell* 2011, **23**:4234-4240.

46. Chen L-Q, Hou B-H, Lalonde S, Takanaga H, Hartung ML, Qu X-Q, Guo W-J, Kim J-G, Underwood W, Chaudhuri B *et al.*: **Sugar transporters for intercellular exchange and nutrition of pathogens**. *Nature* 2010, **468**:527-532.

47. Rolland F, Baena-Gonzalez E, Sheen J: **Sugar sensing and signaling in plants. conserved and novel mechanisms**. *Annu Rev Plant Biol* 2006, **57**:675-709.

48. Xiong Y, McCormack M, Li L, Hall Q, Xiang C, Sheen J: **Glucose-TOR signalling reprograms the transcriptome and activates meristems**. *Nature* 2013, **496**:181-186.

49. Mason MG, Ross JJ, Babst BA, Wienclaw BN, Beveridge CA: **Sugar demand, not auxin, is the initial regulator of apical dominance**. *Proc Natl Acad Sci USA* 2014, **111**:6092-6097.

50. Lastdrager J, Hanson J, Smeekens S: **Sugar signals and the control of plant growth and development**. *J Exp Bot* 2014, **65**:799-807.

51. Ruan Y-L: **Sucrose metabolism: gateway to diverse carbon use and sugar signaling**. *Annu Rev Plant Biol* 2014, **65**:33-67.

52. Borisjuk L, Rolletschek H, Radchuk R, Weschke W, Wobus U, Weber H: **Seed development and differentiation: a role for metabolic regulation**. *Plant Biol (Stuttg)* 2004, **6**:375-386.

53. Teslaa T, Teitell MA: **Pluripotent stem cell energy metabolism: an update**. *EMBO J* 2015, **34**:138-153.

54. Rafalski VA, Mancini E, Brunet A: **Energy metabolism and energy-sensing pathways in mammalian embryonic and adult stem cell fate**. *J Cell Sci* 2012, **125**:5597-5608.

55. Rathbone CR, Booth FW, Lees SJ: **Sirt1 increases skeletal muscle precursor cell proliferation**. *Eur J Cell Biol* 2009, **88**:35-44.

56. Fulco M, Schiltz RL, Iezzi S, King MT, Zhao P, Kashiwaya Y, Hoffman E, Veech RL, Sartorelli V: **Sir2 regulates skeletal muscle differentiation as a potential sensor of the redox state**. *Mol Cell* 2003, **12**:51-62.

57. Hashida S-N, Takahashi H, Uchimiya H: **The role of NAD biosynthesis in plant development and stress responses**. *Ann Bot* 2009, **103**:819-824.

58. Zhao Y, Hu Q, Cheng F, Su N, Wang A, Zou Y, Hu H, Chen X, Zhou H-M, Huang X *et al.*: **SoNar, a highly responsive NAD(+)/NADH sensor, allows high-throughput metabolic screening of anti-tumor agents**. *Cell Metab* 2015, **21**:777-789.

59. Hung YP, Albeck JG, Tantama M, Yellen G: **Imaging cytosolic NADH-NAD(+) redox state with a genetically encoded fluorescent biosensor**. *Cell Metab* 2011, **14**:545-554.

60. Zhao Y, Jin J, Hu Q, Zhou H-M, Yi J, Yu Z, Xu L, Wang X, Yang Y, Loscalzo J: **Genetically encoded fluorescent sensors for intracellular NADH detection**. *Cell Metab* 2011, **14**:555-566.

61. Imamura H, Nhat KPH, Togawa H, Saito K, Iino R, Kato-Yamada Y, Nagai T, Noji H: **Visualization of ATP levels inside single living cells with fluorescence resonance energy transfer-based genetically encoded indicators**. *Proc Natl Acad Sci USA* 2009, **106**:15651-15656.

62. Nakano M, Imamura H, Nagai T, Noji H: **Ca^{2+} regulation of mitochondrial ATP synthesis visualized at the single cell level**. *ACS Chem Biol* 2011, **6**:709-715.

63. Hardie DG, Ross FA, Hawley SA: **AMPK: a nutrient and energy sensor that maintains energy homeostasis**. *Nat Rev Cancer* 2012, **13**:251-262.

64. Thelander M, Olsson T, Ronne H: **Snf1-related protein kinase 1 is needed for growth in a normal day–night light cycle**. *EMBO J* 2004, **23**:1900-1910.

65. Tantama M, Martínez-François JR, Mongeon R, Yellen G: **Imaging energy status in live cells with a fluorescent biosensor of the intracellular ATP-to-ADP ratio**. *Nat Commun* 2013, **4**:2550.

66. Berg J, Hung YP, Yellen G: **A genetically encoded fluorescent reporter of ATP:ADP ratio**. *Nat Methods* 2009, **6**:161-166.

67. Tanaka K, Gilroy S, Jones AM, Stacey G: **Extracellular ATP signaling in plants**. *Trends Cell Biol* 2010, **20**:601-608.

68. Reichler SA, Torres J, Rivera AL, Cintolesi VA, Clark G, Roux SJ: **Intersection of two signalling pathways: extracellular nucleotides regulate pollen germination and pollen tube growth via nitric oxide**. *J Exp Bot* 2009, **60**:2129-2138.

69. Gilroy S, Suzuki N, Miller G, Choi W-G, Toyota M, Devireddy AR, Mittler R: **A tidal wave of signals: calcium and ROS at the forefront of rapid systemic signaling**. *Trends Plant Sci* 2014, **19**:623-630.

70. Choi W-G, Swanson SJ, Gilroy S: **High-resolution imaging of Ca^{2+}, redox status, ROS and pH using GFP biosensors**. *Plant J* 2012, **70**:118-128.

71. Schmidt R, Schippers JHM: **ROS-mediated redox signaling during cell differentiation in plants**. *Biochim Biophys Acta* 2015, **1850**:1497-1508.

72. Wang W, Fang H, Groom L, Cheng A, Zhang W, Liu J, Wang X, Li K, Han P, Zheng M *et al.*: **Superoxide flashes in single mitochondria**. *Cell* 2008, **134**:279-290.

73. Belousov VV, Fradkov AF, Lukyanov KA, Staroverov DB, Shakhbazov KS, Terskikh AV, Lukyanov S: **Genetically encoded fluorescent indicator for intracellular hydrogen peroxide**. *Nat Methods* 2006, **3**:281-286.

74. Costa A, Drago I, Behera S, Zottini M, Pizzo P, Schroeder JI, Pozzan T, Schiavo Lo F: **H_2O_2 in plant peroxisomes: an in vivo analysis uncovers a Ca^{2+}-dependent scavenging system**. *Plant J* 2010, **62**:760-772.

75. • Ermakova YG, Bilan DS, Matlashov ME, Mishina NM, Markvicheva KN, Subach OM, Subach FV, Bogeski I, Hoth M, Enikolopov G *et al.*: **Red fluorescent genetically encoded indicator for intracellular hydrogen peroxide**. *Nat Commun* 2014, **5**:5222.

Visualizing reactive oxygen species in live tissue has been a challenging task, in particular due to the difficulty of measuring other molecules simultaneously. Here the authors present a novel red variant of HyPer, which is specific to H_2O_2 and allows co-expression of sensors that can help identify co-regulated substrates or act as controls.

76. Geigenberger P: **Response of plant metabolism to too little oxygen**. *Curr Opin Plant Biol* 2003, **6**:247-256.

77. Potzkei J, Kunze M, Drepper T, Gensch T, Jaeger K-E, Büchs J: **Real-time determination of intracellular oxygen in bacteria using a genetically encoded FRET-based biosensor**. *BMC Biol* 2012, **10**:28.

78. Sondergaard TE, Schulz A, Palmgren MG: **Energization of transport processes in plants. roles of the plasma membrane H^+-ATPase**. *Plant Physiol* 2004, **136**:2475-2482.

79. Schumacher K: **pH in the plant endomembrane system — an import and export business**. *Curr Opin Plant Biol* 2014, **22**:71-76.

80. Bak G, Lee E-J, Lee Y, Kato M, Segami S, Sze H, Maeshima M, Hwang J-U, Lee Y: **Rapid structural changes and acidification of guard cell vacuoles during stomatal closure require phosphatidylinositol 3,5-bisphosphate**. *Plant Cell* 2013, **25**:2202-2216.

81. Bassil E, Coku A, Blumwald E: **Cellular ion homeostasis: emerging roles of intracellular NHX Na^+/H^+ antiporters in plant growth and development**. *J Exp Bot* 2012, **63**:5727-5740.

82. Li J, Yang H, Peer WA, Richter G, Blakeslee J, Bandyopadhyay A, Titapiwantakun B, Undurraga S, Khodakovskaya M, Richards EL *et al.*: **Arabidopsis H^+-PPase AVP1 regulates auxin-mediated organ development**. *Science* 2005, **310**:121-125.

83. Viotti C, Krüger F, Krebs M, Neubert C, Fink F, Lupanga U, Scheuring D, Boutté Y, Frescatada-Rosa M, Wolfenstetter S *et al.*: **The endoplasmic reticulum is the main membrane source for biogenesis of the lytic vacuole in Arabidopsis**. *Plant Cell* 2013, **25**:3434-3449.

84. Martinière A, Desbrosses G, Sentenac H, Paris N: **Development and properties of genetically encoded pH sensors in plants**. *Front Plant Sci* 2013, **4**:523.

85. Martinière A, Bassil E, Jublanc E, Alcon C, Reguera M, Sentenac H, Blumwald E, Paris N: **In vivo intracellular pH measurements in tobacco and Arabidopsis reveal an unexpected pH gradient in the endomembrane system**. *Plant Cell* 2013, **25**:4028-4043.

86. Hellwig N, Plant TD, Janson W, Schäfer M, Schultz G, Schaefer M: **TRPV1 acts as proton channel to induce acidification in nociceptive neurons**. *J Biol Chem* 2004, **279**:34553-34561.

87. •• Luo Yu, Scholl S, Doering A, Zhang Y, Irani Niloufer G, Rubbo Simone Di, Neumetzler L, Krishnamoorthy P, Houtte Isabelle Van, Mylle Evelien *et al.*: **V-ATPase activity in the TGN/EE is required for exocytosis and recycling in Arabidopsis**. *Nat Plants* 2015, **1**:1-10.

An elegant study, using the ratiometric sensor pHusion for measuring pH inside and outside of TGN/early endosomes; unveiling the importance of acidification for secretion and recycling.

88. Gjetting KSK, Ytting CK, Schulz A, Fuglsang AT: **Live imaging of intra- and extracellular pH in plants using pHusion, a novel genetically encoded biosensor**. *J Exp Bot* 2012, **63**:3207-3218.

89. Vanstraelen M, Benková E: **Hormonal interactions in the regulation of plant development**. *Annu Rev Cell Dev Biol* 2012, **28**:463-487.

90. Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ: **Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements**. *Plant Cell* 1997, **9**:1963-1971.

91. Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, Burrow AH, Beeckman T, Kepinski S, Traas J, Bennett MJ *et al.*: **A novel sensor to map auxin response and distribution at high spatio-temporal resolution**. *Nature* 2012, **482**:103-106.

92. •• Liao C-Y, Smet W, Brunoud G, Yoshida S, Vernoux T, Weijers D: **Reporters for sensitive and quantitative measurement of auxin response**. *Nat Methods* 2015, **12**:207-210.

The tools developed in this work represent an advanced set of auxin reporters. The reporters are modified transcriptional and degron-based reporters that now allow ratiometric measurements.

93. Löfke C, Zwiewka M, Heilmann I, Van Montagu MCE, Teichmann T, Friml J: **Asymmetric gibberellin signaling regulates vacuolar trafficking of PIN auxin transporters during root gravitropism**. *Proc Natl Acad Sci USA* 2013, **110**:3627-3632.

94. Mertens RD, Weiler EW: **Kinetic studies on the redistribution of endogenous growth regulators in gravireacting plant organs**. *Planta* 1983, **158**:339-348.

95. Brock TG, Kaufman PB: **Altered growth response to exogenous auxin and gibberellic acid by gravistimulation in pulvini of *Avena sativa***. *Plant Physiol* 1988, **87**:130-133.

96. Hentrich M, Böttcher C, Düchting P, Cheng Y, Zhao Y, Berkowitz O, Masle J, Medina J, Pollmann S: **The jasmonic acid signaling pathway is linked to auxin homeostasis through the modulation of YUCCA8and YUCCA9gene expression**. *Plant J* 2013, **74**:626-637.

97. Wasternack C, Hause B: **Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany**. *Ann Bot* 2013, **111**:1021-1058.

98. • Larrieu A, Champion A, Legrand J, Lavenus J, Mast D, Brunoud G, Oh J, Guyomarc'h S, Pizot M, Farmer EE *et al.*: **A fluorescent hormone biosensor reveals the dynamics of jasmonate signalling in plants**. *Nat Commun* 2015, **6**:6043.

Development of a reporter for the hormone jasmonic acid that is based on reporter degradation — similar concept as for the auxin reporter DII-Venus. The reporter allowed to visualize long-distance signaling in roots after wounding of cotelydons.

99. Finkelstein R: **Abscisic acid synthesis and response**. *Arabidopsis Book* 2013, **11**:e0166.

100. Jones AM: **A new look at stress: abscisic acid patterns and dynamics at high-resolution**. *New Phytol* 2015 http://dx.doi.org/10.1111/nph.13552.

101. Finkelstein RR, Somerville CR: **Three classes of abscisic acid (ABA)-insensitive mutations of arabidopsis define genes that control overlapping subsets of ABA responses**. *Plant Physiol* 1990, **94**:1172-1179.

102. Xiong L, Zhu J-K: **Regulation of abscisic acid biosynthesis**. *Plant Physiol* 2003, **133**:29-36.

103. •• Waadt R, Hitomi K, Nishimura N, Hitomi C, Adams SR, Getzoff ED, Schroeder JI, Amasino R: **FRET-based reporters for the direct visualization of abscisic acid concentration changes and distribution in Arabidopsis**. *eLife* 2014:3.

This reference along with Ref. [104••] developed sensors for ABA, the first available directly binding phytohormone sensors.

104. •• Jones AM, Danielson JÅ, Manojkumar SN, Lanquar V, Grossmann G, Frommer WB: **Abscisic acid dynamics in roots detected with genetically encoded FRET sensors**. *eLife* 2014, **3**:e01741.

See annotation to Ref. [103••].

105. Schwarzländer M, Wagner S, Ermakova YG, Belousov VV, Radi R, Beckman JS, Buettner GR, Demaurex N, Duchen MR, Forman HJ *et al.*: **The ''mitoflash'' probe cpYFP does not respond to superoxide**. *Nature* 2014, **514**:E12-E14.

106. Borst JW, Willemse M, Slijkhuis R, van der Krogt G, Laptenok SP, Jalink K, Wieringa B, Fransen JAM: **ATP changes the fluorescence lifetime of cyan fluorescent protein via an interaction with His148**. *PLoS ONE* 2010, **5**:e13862.

107. Jones AM, Grossmann G, Danielson JÅ, Sosso D, Chen L-Q, Ho C-H, Frommer WB: ***In vivo* biochemistry: applications for small molecule biosensors in plant biology**. *Curr Opin Plant Biol* 2013, **16**:389-395.

108. Lanquar V, Grossmann G, Vinkenborg JL, Merkx M, Thomine S, Frommer WB: **Dynamic imaging of cytosolic zinc in Arabidopsis roots combining FRET sensors and RootChip technology**. *New Phytol* 2013, **202**:198-208.

109. • De Michele R, Ast C, Loqué D, Ho C-H, Andrade SL, Lanquar V, Grossmann G, Gehne S, Kumke MU, Frommer WB *et al.*: **Fluorescent sensors reporting the activity of ammonium transceptors in live cells**. *eLife* 2013:2.

Instead of avoiding any interference by the biosensor in the measured process this study used a catalytically active sensor to reveal activity and conformational changes of an enzyme.

110. Ho C-H, Frommer WB: **Fluorescent sensors for activity and regulation of the nitrate transceptor CHL1/NRT1.1 and oligopeptide transporters**. *eLife* 2014, **3**:e01917.

111. Gu H, Lalonde S, Okumoto S, Looger LL, Scharff-Poulsen AM, Grossman AR, Kossmann J, Jakobsen I, Frommer WB: **A novel analytical method for in vivo phosphate tracking**. *FEBS Lett* 2006, **580**:5885-5893.

112. Mukherjee P, Banerjee S, Wheeler A, Ratliff LA, Irigoyen S, Garcia LR, Lockless SW, Versaw WK: **Live imaging of inorganic phosphate in plants with cellular and subcellular resolution**. *Plant Physiol* 2015, **167**:628-638.