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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

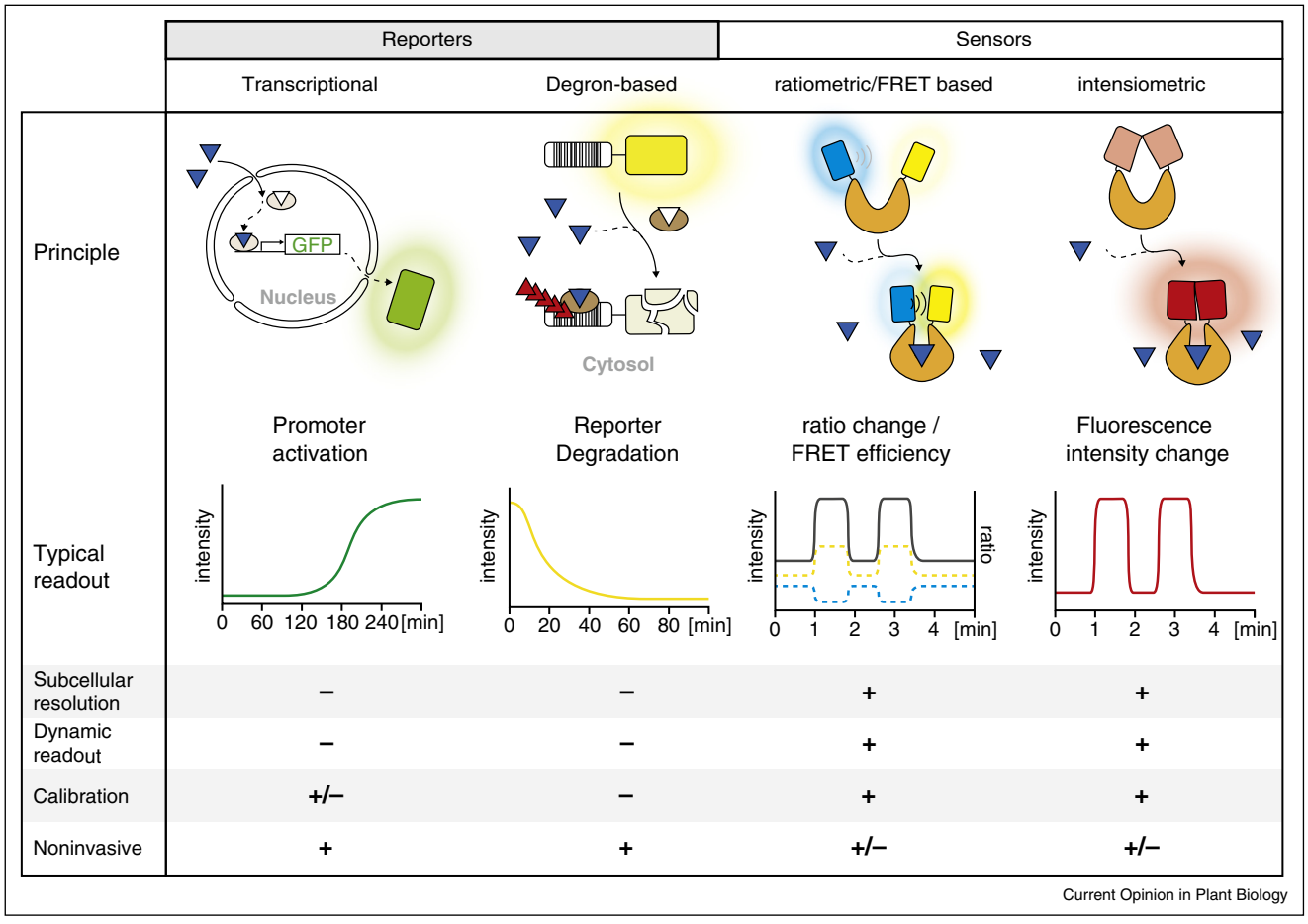
Growing genetically identical plants under different environmental conditions results in different plant morphologies. This widely known fact highlights the ability of plants for plastic adaptation of the body plan to meet the requirements of a sessile lifestyle. Anatomical adaptations can be observed 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