Mobility of signaling molecules: the key to deciphering plant organogenesis

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Abstract Signaling molecules move between cells to form a characteristic distribution pattern within a developing organ; thereafter, they spatiotemporally regulate organ development. A key question in this process is how the signaling molecules robustly form the precise distribution on a tissue scale in a reproducible manner. Despite of an increasing number of quantitative studies regarding the mobility of signaling molecules, the detail mechanism of organogenesis via intercellular signaling is still unclear. We here review the potential advantages of plant development to address this question, focusing on the cytoplasmic continuity of plant cells through the plasmodesmata. The plant system would provide a unique opportunity to define the simple transportation mode of diffusion process, and, hence, the mechanism of organogenesis via intercellular signaling. Based on the advances in the understanding of intercellular signaling at the molecular level and in the quantitative imaging techniques, we discuss our current challenges in measuring the mobility of signaling molecules for deciphering plant organogenesis.

Keywords Intercellular signaling · Morphogen gradient · Plant organogenesis · Protein diffusion dynamics

Introduction

A developing organ comprises a heterogeneous field of individual cells that differ in terms of physical conditions, molecular constitution, structures, differentiation stages and other properties. Cells orchestrate such variables using cell-to-cell communication to achieve normal organogenesis. The movement of signaling molecules between cells defines their spatiotemporal distribution within a developing organ, and, thereafter, guides developmental progression. Thus, quantitative characterization of the movement of signaling molecules would facilitate understanding of the mechanism of organogenesis. The kinetics of intercellular signaling is being investigated in animal development, using quantitative imaging techniques such as fluorescence redistribution (or recovery) after photobleaching (FRAP) and photoconversion of fluorescent proteins (reviewed in Kicheva et al. 2012; Müller et al. 2013; Wartlick et al. 2009). Pioneer studies have reported that signaling molecules are transported in a non-directed manner and that their tissue-scale distribution can be explained, at least in part, by the kinetics of their movement (Kicheva et al. 2007; Müller et al. 2012; Yu et al. 2009).

The movement of signaling molecules between cells is essential for normal organogenesis in plants, as well as in animals. We showed recently that the transcriptional co-activator *ANGUSTIFOLIA3* (*AN3*, which is also known as *GRF-INTERACTING FACTOR1*) (Horiguchi et al. 2005; Kim and Kende 2004) is produced within mesophyll cells of leaf primordia in *Arabidopsis thaliana* (hereafter, *Arabidopsis*), and then moves into epidermal cells (Kawade et al. 2013). Interference with *AN3* movement causes defective proliferation of epidermal cells, resulting in abnormal leaf size and shape (Kawade et al. 2013). A series of studies have identified various signaling

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molecules including AN3, and revealed their functional significance in plant organogenesis (e.g. Jackson et al. 1994; Kawade et al. 2013; Kim et al. 2003; Nakajima et al. 2001). However, our quantitative understanding of the spatiotemporal dynamics of intercellular signaling in plants is limited.

This review aims to highlight the importance of quantitative investigation of signaling molecule mobility to increase our knowledge of plant organogenesis. Quantitative analysis of the movement of signaling molecules in plants can be a promising target because of the simple transportation mode; *i.e.* via the plasmodesmata. Also, comparison of quantitative information of intercellular signaling between animals and plants would enhance our understanding of the similarities and differences in their development. We will firstly see how the tissue-scale distribution of signaling molecules is generated by their intercellular movement using a simple mathematical model. We will next describe the features of intercellular signaling in plants, focusing on plasmodesmata to emphasize the advantages of plant development systems for investigation of the dynamics of intercellular signaling. Lastly, given the advances in the molecular understanding of plant development including AN3 signaling, we will discuss current challenges in quantitative assessment of signaling molecule dynamics during plant organogenesis.

Tissue-scale distribution of signaling molecules via diffusion process

Diffusion of signaling molecules has long been proposed as a primary mechanism of establishment of a tissue-scale gradient distribution (Crick 1970; Turing 1952; Wolpert 1969). Quantitative studies revealed the contribution of diffusion process for morphogen gradient formation in animal development (reviewed in Mülle et al. 2013). Mathematically, diffusion is defined as a process in which movement of materials in a given time is proportional to the concentration difference between two neighbor positions (Berg 1993). The simplest example is random motion of small particles in water (*i.e.* Brownian motion, Brown 1828), which is passively driven by thermal fluctuations of water molecules. Movement of signaling molecules in biological processes through transcytosis and facilitated or suppressed transportation through interaction with other molecules can also be described as diffusion if it satisfies the above conditions.

To assess the mechanism by which signaling molecules form a tissue-scale gradient distribution through the diffusion process, let us consider a simple one-dimensional mathematical model [Synthesis, Diffusion and Degradation (SDD) model (Crick 1970), Fig. 1]. Signaling molecules are constantly produced at a boundary of a tissue ($x = 0$).

Fig. 1 Gradient formation in the SDD model. **a** Time-evolution of distribution of signaling molecules is depicted using the SDD model. Equation 1 is numerically solved with parameters of $D = 3 \mu\text{m}^2 \text{s}^{-1}$ and $k_d = 3.2 \times 10^{-3}$, which yields $\lambda = 30 \mu\text{m}$. Colours indicate dif-

ferent time points. **b** Relationship between gradient pattern and diffusion coefficient. Molecules that more rapidly form a broader distribution. $D = 0.3, 3$ and $30 \mu\text{m}^2 \text{s}^{-1}$ (left to right)

They then spread into the entire tissue ($x > 0$) by diffusion in association with constant degradation. These three processes can be modeled by a reaction–diffusion equation,

$$\frac{\partial C(x, t)}{\partial t} = D \frac{\partial^2 C(x, t)}{\partial x^2} - k_d C(x, t). \quad (1)$$

$C(x, t)$ denotes the concentration of signaling molecule at position x and time t . The first term at the right hand side is the transportation of signaling molecules by diffusion, where D is a diffusion coefficient (unit: $\text{length}^2 \text{time}^{-1}$). The second term is the spatially uniform degradation of signaling molecules with a degradation rate k_d (time^{-1}). This equation should be complemented by two boundary conditions at $x = 0$ and $x = \infty$. The first condition is determined by the assumption that signaling molecules are constantly produced at the left boundary ($x = 0$) with a rate J . This condition is expressed as

$$\left. \frac{\partial C(x, t)}{\partial x} \right|_{x=0} = -\frac{J}{D}, \quad (2)$$

where the production J is balanced by the diffusive flux $-D \partial_x C$. For the second boundary condition at $x = \infty$, we assume that the tissue is so large that it can be approximated as a semi-infinite system. The second condition is expressed as

$$\left. \frac{\partial C(x, t)}{\partial x} \right|_{x=\infty} = 0. \quad (3)$$

Equations 1–3 describe how the concentration of signaling molecules C changes over time (Berezhevskii et al. 2010; Bergmann et al. 2007). Since there is no signaling molecule at $t = 0$, $C(x, t = 0) = 0$ for any x (Fig. 1a, blue line). Signaling molecules are produced and move into an entire tissue at $t > 0$, and then approach to a steady state (from blue to red lines in Fig. 1a) where the concentration does not change in time as $\partial_t C(x, t) = 0$. This final profile $\tilde{C}(x, t)$ can be therefore obtained by setting Eq. 1 = 0,

$$D \frac{d^2 \tilde{C}(x, t)}{dx^2} - k_d \tilde{C}(x, t) = 0. \quad (4)$$

Equation 4 is not a partial differential equation anymore but a second order ordinary differential equation which has only one variable x . Equation 4 indicates that the steady state distribution of signaling molecules show an exponential decay from the production site as

$$\tilde{C}(x, t) = C_0 e^{-x/\lambda}, \quad (5)$$

$$\lambda = \sqrt{\frac{D}{k_d}}. \quad (6)$$

C_0 is the concentration at the left boundary, and λ is the characteristic length at which the concentration decreases

by a factor of e , namely, $\tilde{C}(x_0 + \lambda, t) = \frac{1}{e} \tilde{C}(x_0, t)$ for any x_0 (e is the base of the natural logarithm, ~ 2.7). It is straight forward to check that Eq. 5 combined with Eq. 6 is the solution of Eq. 4, and that this solution obviously satisfies the boundary condition at $x = \infty$ (Eq. 3). We can determine C_0 so that the concentration profile also satisfies the boundary condition at $x = 0$. By using Eqs. 2 and 5, C_0 is determined as follows

$$C_0 = \frac{J}{\sqrt{D \cdot k_d}}. \quad (7)$$

Note that we here neglect the effects of cell movement/growth/division on the gradient formation, and treat that transportation of signaling molecules rely on only movement between cells. Caution is required here that recent studies reported a relationship between morphogen distribution and tissue growth, suggesting that this assumption is not always validated (Averbukh et al. 2014; Wartlick et al. 2014).

Such an exponential gradient of signaling molecules has been observed in several developmental systems, including Bicoid in *Drosophila* syncytial ($\lambda = 80$ – $100 \mu\text{m}$) (Gregor et al. 2005; Houchmandzadeh et al. 2002); Decapentaplegic (Dpp) and Wingless in *Drosophila* wing disks ($\lambda = 20$ and $6 \mu\text{m}$, respectively) (Kicheva et al. 2007); and Fibroblast growth factor 8 in a zebrafish gastrulating embryo ($\lambda = 200 \mu\text{m}$) (Yu et al. 2009). These observations indicate that the in vivo movement of signaling molecules can be described as a diffusion process.

Equations 5, 6 and 7 show how signaling molecule mobility determines their steady-state distribution. When the signaling molecules move faster (namely, the diffusion coefficient D is larger), the characteristic length λ increases, resulting in a broader distribution of signaling molecules (Fig. 1b). In contrast, we can see a steeper distribution of signaling molecules when λ is smaller due to slower movement (Fig. 1b). The degradation rate of signaling molecules, k_d , also affects the shape of the gradient; this is well described in literatures (Dorocco et al. 2011; Inomata et al. 2008, 2013). These studies reported that perturbation on protein stability influences the distribution pattern of morphogen, in consistent with the idea in SDD model (Dorocco et al. 2011; Inomata et al. 2008, 2013). Such perturbation experiments are helpful to know the mechanism for morphogen gradient formation, however, to the best of our knowledge, experiment in which diffusion coefficient of morphogen is artificially changed has not yet been reported.

Quantitative investigation of signaling molecule mobility

Mobility of signaling molecules can be investigated quantitatively by the FRAP assay. In a FRAP experiment,

fluorescently-labeled morphogens in a region of interest are irreversibly photobleached by a strong laser, followed by measurement of fluorescence recovery over time. The curve of fluorescence recovery contains information on the mobility of the molecule analyzed. For example, Dpp fused with green fluorescent protein (GFP) in a *Drosophila* wing imaginal disk has a diffusion coefficient $D = 0.1 \pm 0.05 \mu\text{m}^2 \text{s}^{-1}$ (Kicheva et al. 2007), which is 1000-fold smaller than GFP in water ($D = \sim 100 \mu\text{m}^2/\text{s}$, Terry et al. 1995). Small mobility mediated by non-directed transcytosis involving recurrent cellular uptake and release is proposed to be a key event in the formation of a Dpp gradient (Kicheva et al. 2007).

It is difficult to fully elucidate the mechanism controlling the mobility of signaling molecules because of complex underlying processes including extracellular diffusion, cellular uptake, transcytosis, cell movement and direct delivery via cytonemes (cellular projections of animal cells) (Hsiung et al. 2005; Kornberg 2012; Mülle et al. 2013; Pfeiffer et al. 2000; Ramirez-Weber and Kornberg 1999; Zhou et al. 2012). Indeed, a recent study found that the diffusion coefficient of GFP-Dpp increases tenfold when analyzing the FRAP data by the SDD model with slight modification, taking into consideration diffusion in the extracellular matrix and cellular uptake (Zhou et al. 2012). The tissue geometry with membrane invaginations can make the diffusion process anomalous (Daniels et al. 2012), suggesting that mobility of signalling molecule varies depending on also spatial scales (Abu-Arish et al. 2010; Castle et al. 2011; Gregor et al. 2007).

We have seen that mobility of signaling molecules can determine the characteristic distribution on tissue scale, and that the mobility has been determined in several animal systems. It is no doubt that these studies pioneer a new field to investigate the mechanisms that mediate the morphogen gradient formation, however, the mechanism is not yet fully characterized. The time scale of the FRAP recovery curve is not simply related to that of signaling molecules' mobility, and measurements at different spatial scales often cause fluctuation of the diffusion coefficient. These problems arise from the complex transportation mechanism of signaling molecules and the tissue geometry in animal development. To explain the distribution of signaling molecule, simple experimental systems are required. In the latter part of this review, we will highlight the potential advantages of plant developmental systems.

Plasmodesmata, a unique structure in a plant cell

Plant developmental systems have some potential advantages to quantitatively assess the intercellular signaling dynamics with which we can overcome the difficulties described above. A notable structure of plant cells in terms

of intercellular signaling is the plasmodesmata, which provides cytoplasmic continuity between neighboring cells (reviewed in Brunkard et al. 2013; Burch-Smith and Zambryski 2012; Xu and Jackson 2010). Some nutrients, such as sucrose, RNAs and proteins, can pass through the plasmodesmata, facilitating communication between cells. This direct connection between plant cells means that the effects of cell movement on the distribution of signaling molecules are negligible in plant development, namely plant organogenesis takes place without cell movement. This plant-specific architecture is highly dynamic during development. The plasmodesmata forms a simple structure in young emerged leaves, whereas it becomes more complex in older leaves (Fitzgibbon et al. 2013; Oparka et al. 1999). Protein mobility is also altered in association with this structural change (Oparka et al. 1999), hence regulation of the number and structure of plasmodesmata are critical mechanisms for plant organogenesis via intercellular signaling.

The influences of the aperture size of plasmodesmata on the mobility of signaling molecules have been experimentally examined by monitoring movement of fluorescent probes between plant cells, and been mathematically investigated (Dölger et al. 2014; Liesche and Schulz 2013; Terry and Robards 1987). To estimate the influence of the simple plasmodesmata on the mobility of signaling molecules, first, we consider a situation that the dwell time of the particle in plasmodesmata is shorter than the characteristic time scale of intercellular diffusion. Under this situation, diffusion coefficient at the cell boundary is the same as the permeability across the boundary (Paine et al. 1975). If the aperture is infinitely large, the permeability of signaling molecule is equal to the diffusion coefficient in cytoplasm, which we call D_0 . If the aperture is smaller than size of a molecule, there is no transportation, meaning that the permeability is zero. To consider the effects of finite aperture size on the permeability, we model a signaling molecule as a sphere with a radius a , and plasmodesmata as a hollow cylinder with a radius r . There are two hindrance effects of plasmodesmata that reduce the permeability: (1) molecules hitting the aperture edge are reflected and does not enter into the plasmodesmata, and (2) effective viscosity increases due to the presence of plasmodesmata wall. (1) is purely geometrical effect and is calculated as a probability F_1 that an particle approaching to an aperture does not hit the edge,

$$F_1 = \frac{\pi(r-a)^2}{\pi r^2} = \left(1 - \frac{a}{r}\right)^2. \quad (8)$$

Second factor F_2 is more complicated, however, can be estimated as an increase of hydrodynamic drag force on a particle moving in a center of a cylinder filled with stationary Newtonian fluid (Pain and Scherr 1975). In this case, F_2 is approximately given as

$$F_2 \sim \frac{1 - 0.75857(q_r)^5}{1 - 2.1050(q_r) + 2.0865(q_r)^3 - 1.7068(q_r)^5 + 0.72603(q_r)^6} \quad (9)$$

(Haberman and Sayre 1958). Taken together, the effects of plasmodesmata aperture on diffusion coefficient is

$$\frac{D}{D_0} = F_1 \times F_2 = \left(1 - \frac{a}{r}\right)^2 \times \frac{1 - 0.75857(q_r)^5}{1 - 2.1050(q_r) + 2.0865(q_r)^3 - 1.7068(q_r)^5 + 0.72603(q_r)^6} \quad (10)$$

Using Eq. 10, we plotted the normalized diffusion coefficient D/D_0 as a function of aperture size r with numerically obtained F_2 (Pain and Scherr 1975) (Fig. 2). Here we used the radius of GFP (~ 2 nm) as the size of signaling molecule (Ormö et al. 1996; Pack et al. 2006). Interestingly, the protein mobility is largely affected even when an aperture size is ten times larger than the protein size, and can be changed by more than two folds by modifying aperture radius from 10 to 40 nm.

Deposition and degradation of callose at the neck of the plasmodesmata largely determine the aperture size and therefore also the movement of signaling molecules (Reviewed in Brunkard et al. 2013; De Storme and Geelen 2014). By modulating the expression levels and pattern of callose synthases and β -1, 3-glucanases, which are involved in callose turnover (e.g. Guseman et al. 2010; Levy et al. 2007), the conductivity can be altered in a tissue-specific manner (Vatén et al. 2011). This technique enabled us to conduct a perturbation experiment on the mobility of

Fig. 2 Relationship between aperture size of plasmodesmata and molecular mobility. Normalized diffusion coefficient of a molecule (2 nm radius) is plotted as a function of radius size of aperture using Eq. 10

signaling molecules (Vatén et al. 2011), and facilitated understanding of the effect of signaling molecule mobility on the formation of its characteristic distribution. As for the transportation of signaling molecules via plasmodesmata, it was also revealed recently that a chaperone complex facilitates protein movement via the plasmodesmata, possibly through protein unfolding and refolding (Xu et al. 2011).

As we have seen, movement of signaling molecules via the plasmodesmata could be used to quantitatively investigate the mobility of signaling molecules. In the next section, we will introduce two non-cell-autonomous signaling molecules, AN3 and UPBEAT1 (UPB1), which exhibit interesting behaviour in plant leaves and roots, respectively (Kawade et al. 2013; Tsukagoshi et al. 2010).

Molecular mechanism of intercellular signaling in plants—AN3 and UPB1

The transition from cell proliferation to post-mitotic cell expansion marks the initial step in cellular differentiation. Spatiotemporal control of this transition within a developing organ is a quintessential feature of organogenesis. In Arabidopsis, mitotic cells are distributed uniformly in young leaf primordia. The proliferation phase is then arrested from the leaf tip to the base region, followed by the beginning of the post-mitotic phase (Andriankaja et al. 2012; Donnelly et al. 1999; Kazama et al. 2010). Dynamics of the boundary of this transition during leaf development have been well described (Andriankaja et al. 2012; Donnelly et al. 1999; Kazama et al. 2010; Nath et al. 2003; Tsukaya 2014; White 2006). The *CINCINNATA* (*CIN*) in *Antirrhinum* and *TCP*-family genes in Arabidopsis form a subclade of the class II *TCP*s (*CIN-TCP*s) and are involved in the progression of the boundary (Efroni et al. 2008; Nath et al. 2003). The *SWI/SNF* chromatin remodelling ATPase *BRAHMA* (*BRM*) was recently shown to interact with *CIN-TCP*s to regulate the expression of their downstream genes, and then control boundary progression (Efroni et al. 2013). In addition to *CIN-TCP*s, also AN3 is recruited into the *SWI/SNF* complex for active proliferation of leaf cells (Vercruyssen et al. 2014). The *SWI/SNF-TCP*s and -AN3 mediated regulation is, therefore, proposed to explain, at a molecular level, how the boundary between cell proliferation and post-mitotic cell expansion is spatiotemporally determined during leaf development. Although this idea is appealing, cell proliferation occurs in a broader area than does expression of AN3 (Fig. 3; Horiguchi et al. 2005). Because AN3 can move between leaf epidermal and mesophyll cells (Kawade et al. 2013), one possible explanation for the spatial difference is that the AN3 protein moves within the same cell layer. It would be interesting to determine whether the spatiotemporal dynamics of AN3

distribution corresponds to the transition from cellular proliferation to differentiation during leaf development. This is to be discussed in the next chapter.

Our knowledge, at the molecular level, of the definition of the boundary between cell proliferation and cell elongation where cellular differentiation begins in roots is more advanced (Breuer et al. 2010; Dello Ioio et al. 2007). A basic helix-loop-helix domain (bHLH) transcription factor known as UPBEAT1 (UPB1) is one of the key components in this process (Tsukagoshi et al. 2010). The *UPB1* promoter is active in cells of the lateral root cap (LRC) near the transition zone and in vascular cells of the differentiation zone (Tsukagoshi et al. 2010). In contrast, when UPB1, fused with GFP, is expressed under the control of the same promoter, GFP signal is observed in all cells in the differentiation zone, and also weakly in the cells of the proliferation zone, perhaps due to the movement of UPB1 (Tsukagoshi et al. 2010). These results, together with the data from a series of transgenic lines expressing UPB1 fused with GFP or with tandem three YFPs, suggest that UPB1 is a signaling molecule originating from the LRC that regulates the transition between cell proliferation and differentiation in roots (Tsukagoshi et al. 2010). Transcriptome, ChIP-chip and chemical treatment experiments have revealed that UPB1 directly controls the expression of peroxidases to modulate the balance of reactive oxygen species (ROS) (Tsukagoshi et al. 2010). Although ROS metabolism is known to be involved in the control of the balance between cell proliferation and differentiation in plants and animals (Ostrakhovitch and Semenikhin 2013; Wang et al. 2013) the underlying mechanism remains to be elucidated.

Identification of UPB1 signaling would stimulate further study of the mechanism linking ROS homeostasis with the transition from cell proliferation to differentiation via intercellular signaling.

Toward quantitative understanding of intercellular signaling in plant organogenesis

Our understanding of the functions of AN3 and UPB1 at the molecular level is increasing, but quantitative data regarding their mobility are lacking. This is also true for other signaling molecules that play an important role in plant development. Whether AN3 forms an expression gradient along the leaf proximal-to-distal axis, related to cell proliferation activity, is unknown. If this is the case, the underlying mechanism via intercellular movement is of great interest. Quantitative characterisation of AN3 signaling, together with theoretical investigation based on the SDD model, is thus essential to determine whether AN3 movement occurs in a non-directional manner, whether AN3 movement between cells is mediated simply by diffusion, whether diffusion is sufficient to explain AN3 distribution, and whether the kinetics of spatiotemporal dynamics of cell proliferation are consistent with the AN3-signaling dynamics. To address these issues, some essential parameters could experimentally be determined through FRAP assays where the size of the region of bleaching is modified from single cell to tissue scales (Kawade et al. unpublished): when we photobleach at single cell level, diffusion coefficient and mode of diffusivity between individual cells can be characterized. We can assess directionality and tissue-scale kinetics of protein mobility by analyzing FRAP data with larger region for bleaching, for instance, we may observe direction-dependent signal recovery, which is a sign of directional transportation. The intracellular mobile fraction of AN3 is also interesting, as AN3 is localised mainly to the nucleus and, to a lesser extent, the cytoplasm (Kawade et al. 2010, 2013). Recovery curve of FRAP assay at tissue scale include intermixed information about intracellular and intercellular mobility of signaling molecule (Sprague and McNally 2005). Together, to precisely know the AN3-signaling dynamics within a developing organ, we have to distinguish these mobility kinetics by comparing data from single-cell and tissue-scale FRAP assays. AN3-signaling dynamics can be recapitulated by integrating these parameters in SDD model for further investigation of relationship to cell proliferation dynamics. Similar questions are relevant in the case of UPB1. Such information would clarify the mechanism underlying the control of plant organogenesis by intercellular signaling via the plasmodesmata.

Quantitative studies using plant materials could facilitate investigation of the signaling dynamics during plant

Fig. 3 Distribution of proliferating cells and expression pattern of AN3 in the first leaf primordia. A *cyclin-B1;1* promoter- β -glucuronidase (*GUS*) reporter line (*pCYCB1;1::GUS*, Donnelly et al. 1999) and an *AN3* promoter-*GUS* reporter line (*pAN3::GUS*, Horiguchi et al. 2005) were used to visualize proliferating cells and *AN3*-expressing cells, respectively. Leaf primordia are from 6-day-old seedlings. Method for *GUS* staining is described elsewhere (Kawade et al. 2013). Bars 50 μ m

organogenesis. A transmembrane leucine-rich-repeat receptor-like kinase FLAGELLIN SENSITIVE2 (FLS2) is a component of defence signaling against plant pathogens (Gomez-Gomez and Boller 2000). FRAP assay revealed that 75 % of the FLS2 in the plasma membrane diffuse laterally, with $D = 0.34 \pm 0.02 \mu\text{m}^2 \text{s}^{-1}$ (Ali et al. 2007). However, FLS2 transport is slower, with $D = 0.22 \pm 0.01 \mu\text{m}^2 \text{s}^{-1}$, in the presence of flagellin of plant pathogenic bacteria (Ali et al. 2007). This fact suggests that FLS2 signaling is modulated by a change in its mobility in response to bacterial infection. In addition to determine the diffusion coefficient, the FRAP assay is helpful in understanding the mobile intracellular fraction and directionality. Murata et al. (2013) investigated microtubule behaviour during phragmoplast expansion, using FRAP and other imaging techniques, and revealed two types of microtubules, the stable and dynamic. Kitagawa and Fujita (2013) explored protein diffusivity in protonema cells of *Physcomitrella patens* using the photoconversion protein Dendra2 and demonstrated that protein diffusivity in these cells exhibited directional bias. The utility of Dendra2 is confirmed also in *Arabidopsis* (Wu et al. 2011).

Above-mentioned studies focused mainly on protein diffusivity at a small scale (local diffusivity) (Ali et al. 2007; Kitagawa and Fujita 2013; Murata et al. 2013). Our next challenge is to assess the dynamics of signaling molecules at a larger scale (global diffusivity) to explain the mechanisms by which the tissue-scale distribution of signaling molecules is established during organogenesis, and its control of developmental progression. The wealth of quantitative imaging techniques, together with a unique mode of intercellular signaling in plants, will allow us to decipher the puzzle of organogenesis via intercellular signaling.

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