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Show and tell: cell biology of pathogen invasion

Serry Koh and Shauna Somerville

Because the initial stages of pathogen invasion are often confined to a limited number of host cells, measures of host responses that are averaged over attacked and non-attacked cells provide an unsatisfactory view of these events. To identify the earliest and often transient responses to pathogen attack, there is considerable interest in monitoring the subcellular events that occur specifically in living host cells. Recent improvements in live-cell imaging using fluorescent-tagged markers have expanded the scope of the experiments that can be performed. Changes in the subcellular distribution of organelles and of fluorescently tagged proteins can be monitored in real time in living tissues during pathogen attack, and the dynamic nature of such changes across space and over time can be determined. The application of these sensitive imaging methods has extended earlier observations, made with Nomarski microscopy or inferred from static transmission electron micrographs, about the focal accumulation of subcellular organelles at sites of pathogen attack. In addition, recent experiments have demonstrated the focused accumulation and interaction of specific plant proteins at penetration sites, opening a new window on early host responses and raising questions about the underlying plant processes that sense and direct this marshalling of host resources to block pathogen entry.

Addresses
Carnegie Institution, Department of Plant Biology, 260 Panama Street, Stanford, California 94305, USA

Corresponding author: Somerville, Shauna (ssomerville@stanford.edu)

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Introduction

Understanding how plants and pathogens recognize each other and differentiate to establish either a successful or unsuccessful relationship has been a central question in the field of plant–pathogen interactions. Significant advances have been made in the past decade in identifying plant and microbe determinants of the interaction. One aspect that has received relatively little attention, however, is the changes in the distribution of these various components within living plant cells during the course of infection. Newly developed *in vivo* imaging tools can be very useful in elucidating both dynamic plant cell responses and protein–protein interactions among the players. In this review, we introduce newly developed live-cell imaging methods [1–6] and provide some examples from the past three years of how live-cell imaging methods have advanced our understanding of plant responses to pathogen attack.

Looking inside: an overview of live-cell imaging methods

Earlier methods for imaging live cells were reliant on inherent markers to view the responses of host cells to pathogen attack. Snyder and Nicholson [7] were able to monitor the movement of phytoalexin-laden vesicles toward fungal invasion sites in sorghum cells because the phytoalexin was pigmented. Nomarski optics allowed observations of events such as changes in the pattern of cytoplasmic streaming and the movement of larger organelles such as the nucleus to pathogen entry sites. However, detailed observations were limited. Confocal laser scanning microscopy (CLSM) together with fluorescent tags have opened new opportunities to investigate the subcellular responses to microbes in living plant samples in real time [8,9••,10•,11,12•]. An additional advantage of confocal microscopy is that three-dimensional (3-D) reconstructions of subcellular objects are possible, providing new insights into the spatial organization of the components at contact sites (Figure 1b). As an example, real-time monitoring of the arbuscular mycorrhizal fungi infection process in *Medicago truncatula* root epidermal cells expressing green fluorescent protein (GFP)-labeled endoplasmic reticulum (ER) and cytoskeleton led to the discovery of a novel but transient intracellular structure, the pre-penetration apparatus [9••]. This structure formed prior to penetration by hyphae but failed to form in *dmi* (*doesn't make infections*) mutants, which do not support mycorrhizal colonization. These observations only became possible with the development of fluorescent molecules that have improved properties such as photostability, pH independence, small size and increased brightness, and that exhibited a wide range of excitation/emission spectra [4–6]. Recently, Dixit *et al.* [5] and Shaw [6] and have written comprehensive reviews of the opportunities provided by real-time imaging of plant cells using various spectral fluorescent proteins and confocal microscopy. These reviews also provide helpful cautionary notes about the implementation and interpretation of these methods. Some of these methods are described briefly below and are summarized in Table 1.

Table 1

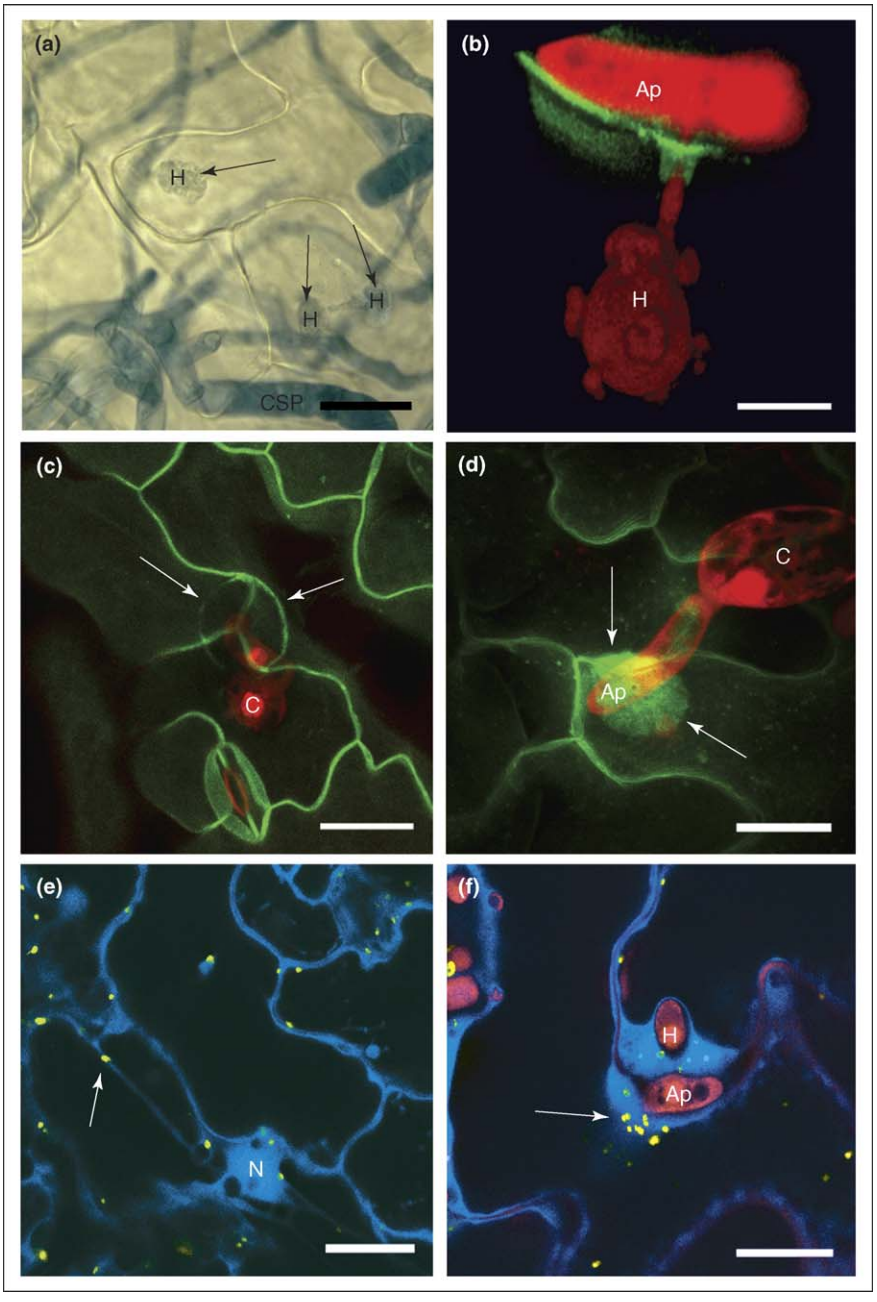
Methods for visualizing protein–protein interactions and dynamics in living cells.

Method	Fluorescent proteins (excitation/emission wavelengths)		Principle	References
	Donor	Acceptor		
Fluorescence resonance energy transfer (FRET)	CFP[a] (440 nm/480 nm)	YFP[b] (480 nm/530 nm)	A light-activated donor molecule emits energy that will subsequently excite an acceptor in close proximity because of protein–protein interaction.	[4,5,22,23••]
Bioluminescence resonance energy transfer (BRET)	RLUC[c] (coelenterazine/480 nm)	YFP (480 nm/530 nm)	Similar to FRET, but uses bioluminescence that is generated by the enzyme luciferase acting on a substrate as a donor light source.	[18,19]
Bimolecular functional complementation (BiFC)	Amino terminus of YFP subfragment (154 amino acids)	Carboxyl terminus of YFP subfragment (83 amino acids)	Reconstitution of a functional fluorophore by close contact of two proteins tagged with subfragments of YFP.	[19–21]
Fluorescence lifetime imaging microscopy (FLIM)	CFP (440 nm/480 nm)	YFP (480 nm/530 nm)	Measures the donor fluorescence lifetime in FRET-based interaction, allowing quantitative detection of interacting proteins in live cells.	[5,23••,24]
FRET–acceptor photobleaching (FRET–APB)	CFP (440 nm/480 nm)	YFP (480 nm/530 nm)	Measures the increase in donor fluorescence after photobleaching the acceptor.	[5,23••]
Fluorescence recovery after photobleaching (FRAP)	Various spectral fluorescent proteins		High-intensity excitation scans in a small region and short time-lapse imaging are used to visualize the recovery rate and distribution pattern of a protein of interest.	[5,23••]
Fluorescence correlation spectroscopy (FCM)	Various spectral fluorescent proteins		Fluctuation of fluorescence intensity of a single molecule in the small confocal observation volume (1 μm^3) can be measured over time as a measure of the mobility of a fluorescently labeled molecule.	[24,26]

[a] CFP, cyan fluorescent protein.
[b] YFP, yellow fluorescent protein.
[c] RLUC, luciferase from *Renilla*.

Using newly developed *in vivo* fluorescence imaging methods, protein–protein interactions can also be visualized in living samples, validating interactions identified by *ex planta* assays such as yeast two-hybrid interaction or co-immunoprecipitation studies [3–6]. The best-known imaging method for studies of protein–protein interactions is fluorescence resonance energy transfer (FRET), which is based on the non-radiative energy transfer from a donor to an acceptor molecule occurring within a distance of about 50 Å. This energy transfer allows the detection of dynamic interactions between two partners *in planta*. Two fluorescent reporter proteins that have different excitation and emission spectra are fused to two proteins of interest. Commonly, cyan fluorescence protein (CFP) and yellow fluorescence protein (YFP) are used, although other combinations are possible [13]. For example, FRET has been used to demonstrate interaction between the VHA-a and VHA-c subunits of vacuolar H^+-ATPase in both *Arabidopsis* and onion protoplasts [14–16]. Intra-protein FRET has been used to quantify metabolites in living plant cells using metabolite sensors that bring CFP and YFP together upon changes in protein conformation that are brought about by binding a metabolite [17]. Bioluminescence resonance energy transfer (BRET) is similar in principle to FRET. BRET makes use of the bioluminescent blue light generated by luciferase from the sea pansy *Renilla* (RLUC) as the donor signal, whereas in FRET, the donor signal is generated by laser activation of a fluorescent CFP molecule. RLUC acts on the substrate coelenterazine, which is nontoxic and membrane permeable [18]. BRET has been used to investigate cellular-signaling events that occur in plants in response to light and the circadian clock [18,19]. For example, the CONSTITUTIVE PHOTOMORPHOGENESIS1 (COP1) leucine-rich nuclear exclusion signal was shown to be necessary for COP1 dimerization and nuclear exclusion using BRET [19]. A third method for imaging protein–protein interactions is bimolecular fluorescence complementation (BiFC). Complementary fragments of YFP (or other fluorescent reporter proteins) are attached to two proteins. If these proteins interact, the two YFP fragments are brought together and a functional YFP is reconstituted [4,20–22]. This method has the advantage that no complicated instrumentation is required to detect protein–protein interactions *in planta*, but suffers from the disadvantage that dynamic changes in such interactions cannot be evaluated. Fluorescence lifetime imaging microscopy (FLIM) is another way to determine protein–

Figure 1



Examples of the confocal laser scanning microscopy imaging of various fluorescently tagged organelles and proteins to monitor dynamic subcellular responses to pathogen attack. **(a)** Conventional trypan blue staining and bright-field microscopy provide limited resolution of the haustorial complex (H, arrows) that is formed within *Arabidopsis* epidermal cells by the virulent *Arabidopsis* powdery mildew pathogen (*E. cichoracearum*). Scale bar represents 40 μm. **(b)** 3-D rendering of a mature *Arabidopsis* powdery mildew haustorium (H) within an *Arabidopsis* epidermal cell. The fungal appressorium (Ap) lies on the plant epidermal cell surface. The plant plasma membrane (green GFP–LTI6a marker) forms a collar around the haustorial neck. Note the absence of labeling of the extrahaustorial membrane, the specialized membrane that encases the haustorium, by this plasma membrane marker. The appressorium and haustorium are stained with propidium iodide (red). Scale bar represents 7 μm. **(c)** At sites of attempted penetration, an early plant response is the accumulation of plasma membrane proteins (illustrated by a GFP–VAMP3 marker) in a ring-like structure (arrows) centered on the penetration site. This large ring can cross cell boundaries as shown here. This is an image of *Arabidopsis* epidermal cells inoculated with the barley powdery mildew (*B. graminis hordei*); similar rings were observed following inoculation with the compatible *Arabidopsis* powdery mildew pathogen (*E. cichoracearum*) [10•]. The conidium (C) and other fungal structures are stained with propidium iodide (red). Scale bar represents 27 μm. **(d)** Focal accumulation of GFP-tagged PEN3/PDR8 ABC transporter (arrows) in *Arabidopsis* epidermal cells inoculated with *E. cichoracearum*. A similar accumulation of PEN3/PDR8–GFP was observed following inoculation with *B. graminis hordei* [39]. The *Arabidopsis* PEN1/SYP121 and its barley homolog ROR2, as well as the barley MLO proteins, accumulate in similar disk-like microdomains following powdery mildew attack [23••,32•]. The fungal conidium and appressorium are stained with propidium iodide (red). Scale bar represents

protein interactions *in vivo*. This FRET-related method relies on measurements of the reduction in the time that the donor fluorescent molecule remains in the excited state in the presence of an acceptor molecule as a measure of protein–protein interaction [5,6,23••,24]. Other variations on FRET, such as FRET-acceptor photobleaching (FRET-APB), exist that are less subject to background fluorescence arising from bleed-through of CFP excitation light into the YFP channel [23••]. Although their application is more technically challenging than FRET, these methods are very sensitive.

A complementary method for observing dynamic changes in proteins or organelles *in planta* is fluorescence recovery after photobleaching (FRAP). In this method, a fluorescent-tagged structure is photobleached with a pulse from a high-energy laser. The recovery of fluorescence at the photobleached site can provide information about the movement of the tagged structure in the cell. As an example, FRAP has been used to monitor the recycling of Golgi-membrane-localized proteins, highlighting the dynamic nature of the Golgi membranes [5,6,23••,25]. Fluorescence correlation microscopy (FCM) can be used to monitor the movement of small numbers of molecules in a defined space, and has been used to monitor binding of the Nod factor to the cell walls of legume root hairs [26].

Interactions among plants and pathogens: working together for a purpose

A frequent observation is that the levels of similar plant transcripts or proteins increase following attack by virulent or avirulent pathogens, but that the increase is delayed in compatible plant–pathogen interactions [27]. This has led to the suggestion that what distinguishes a successful from an unsuccessful pathogen attack are not so much differences in the spectrum of defenses that are elicited but differences in either the sensitivity of the pathogen to the defenses (e.g. phytoalexins, toxic peptides and reactive oxygen species) or the ability of the pathogen to elicit or suppress host defenses [28,29]. From these observations, Thordal-Christensen [30] developed a model of disease resistance in which the plant deploys a series of defensive strategies; inappropriate pathogens cannot defeat the first lines of defense whereas successful pathogens overcome all defenses (see Figure 1 from [30]). In this review, we consider the subcellular plant responses to pathogen attack that can be observed in live cells. We focus on recent observations of *Arabidopsis* plants infected with biotrophic fungi and oomycetes, especially those that attack host epidermal cells, which are readily observed by CLSM.

The first line of defense: penetration resistance

Observational and mutational studies suggest that barriers operating at the cell periphery that prevent invasion represent the first line of defense against pathogens that directly penetrate into plant cells. These can include preformed defenses that depend on the nature and thickness of the waxy outer layer and cuticle or the composition and physical properties of the cell wall. Reinforcement of the cell wall and the deposition of callose-rich papillae at attempted penetration sites are examples of induced defenses at the cell periphery. In addition, the plant may export toxic secondary metabolites and anti-microbial proteins into the apoplast in response to pathogen attack. Once the cell wall defenses are breached, then intracellular defenses assume a greater role in protecting the plant [30].

The best-studied gene controlling penetration resistance, the barley *MLO* gene, suppresses penetration resistance. Natural and induced *mlo* mutants block entry by the powdery mildew pathogen (*Blumeria graminis* f. sp. *hordei*) into barley epidermal cells [31]. Consistent with the role of the cell wall as an important barrier to pathogen entry, the *mur1* mutant, which has walls that have diminished tensile strength, is partially compromised in penetration resistance [32•,33]. Surveys of known defense mutants suggest that *ENHANCED DISEASE SUSCEPTIBILITY1* (*EDS1*) and some of the salicylic acid (SA) pathway genes also play a modest role in penetration resistance to some inappropriate pathogens [29,34,35].

The actin microfilament component of the plant cytoskeleton is important for penetration resistance. Takemoto *et al.* [8] reported that cytoplasmic aggregation and the accumulation of ER and Golgi bodies at penetration sites were associated with active reorganization of actin microfilaments in *Arabidopsis* plants inoculated with *Phytophthora sojae* (an inappropriate pathogen), *Peronospora parasitica* Cala2 (an avirulent pathogen), and *P. parasitica* Noks1 (a virulent pathogen). Penetration by pathogens is required to stimulate this actin reorganization at infection sites; this reorganization is not elicited in penetration-deficient anthracnose (*Colletotrichum lagenarium*) mutants [12•]. Consistent with this observation, pharmacological inhibition of actin polymerization significantly reduced penetration resistance to inappropriate anthracnose sp. in *Arabidopsis*, and severely compromised resistance against

17 μm. **(e)** Two spectrally distinct fluorescent proteins were used to tag the cytoplasm (soluble CFP, shown in blue) and the peroxisomes (YFP–tetrafunctional protein marker, shown in yellow, arrow) in healthy, uninoculated *Arabidopsis* epidermal cells. The peroxisomes are dispersed throughout the cell. Scale bar represents 15.6 μm. **(f)** Peroxisomes (YFP–tetrafunctional protein marker and shown in yellow, arrow) and cytoplasm (soluble CFP, shown in blue) accumulate in an attacked *Arabidopsis* epidermal cell below the *E. cichoracearm* appressorium, adjacent to the fungal haustorium, and in an adjacent cell. The focal accumulation of cytoplasm and organelles is stable in these Latrunculin B-treated cells, suggesting that the actin microfilament network is not required to maintain this distribution. The powdery mildew appressorium and haustorium are stained with propidium iodide (red). Scale bar represents 19.6 μm. Csp, conidiophore; N, plant nucleus.

the wheat powdery mildew (*B. graminis tritici*) in *eds1 Arabidopsis* mutants [12•,34]. Similarly, inhibition of both actin polymerization and, to a lesser extent, inhibition of microtubules increased the penetration success of the pea powdery mildew (*Erysiphe pisi*) on barley [36]. These observations implicate the actin cytoskeleton in the redistribution of subcellular organelles and in penetration resistance.

Several groups conducted mutant screens to identify additional components of penetration resistance. In *Arabidopsis*, the barley powdery mildew is stopped at the penetration phase. Only about 5% of individual spores are able to breach the cell wall and form a haustorial feeding structure in a host epidermal cell, whereas the *Arabidopsis* powdery mildew is able to successfully penetrate the cell wall about 90% of the time [29]. A series of *Arabidopsis penetration* (*pen*) mutants were identified as mutants that allow increased penetration success by the barley powdery mildew pathogen. To date, three *PEN* genes have been characterized: *PEN1* (*SYP121*), which encodes a syntaxin [37]; *PEN2*, encoding a glycosyl hydrolase [38]; and *PEN3* (also called *PLEIOTROPIC DRUG RESISTANCE8* [*PDR8*]), which encodes an ABC transporter [39].

The PEN proteins and MLO protein become redistributed in plant cells upon attack by the powdery mildew pathogens. Previous biochemical studies localized the MLO protein to the plasma membrane [40]. Recent cell biology experiments expand this observation to living tissues by showing that MLO–GFP accumulates in a disc-shaped patch at the sites of powdery mildew invasion [23••]. Using FLIM and FRET-APB, Bhat *et al.* [23••] also showed that calmodulin interacts with MLO within these disc-shaped patches, providing *in planta* confirmation of earlier biochemical studies showing an interaction between MLO and calmodulin [41]. Bhat *et al.* [23••] also show that the barley PEN1/SYP121 homolog, ROR2 (REQUIRED FOR mlo RESISTANCE2), and the *Arabidopsis* PEN1/SYP121 accumulate in similar disc-shaped patches at the sites of powdery mildew attack. Given that barley *ror2* mutations partially suppress the *mlo*-based penetration resistance, this co-accumulation at sites of pathogen attack suggests that these proteins might interact directly with MLO, modulating the ability of the PEN1/SYP121 syntaxin to interact with its partner SNAP (t-SNAREs on the presynaptic membrane) and VAMP (v-SNARE on synaptic vesicles) proteins to mediate targeted vesicle docking and exocytosis at the plasma membrane at sites of attempted pathogen entry [42]. Although predicted to be a soluble protein, the PEN2 glycosyl hydrolase appears to associate with peroxisomes, which aggregate at penetration sites in epidermal cells that have been attacked by powdery mildew [10•,38]. Collectively, these observations highlight the targeted marshalling of resources to sites of attempted pathogen invasion and the importance of defenses that act in the apoplast against biotrophic pathogens, such as the powdery mildews. The hemibiotrophic anthracnose pathogen did not, however, stimulate the accumulation of PEN1–GFP into a microdomain at infection sites, highlighting the need for similar cytological studies of pathogens that have a broad range of infection strategies [12•].

The plasma-membrane-resident PEN3/PDR8 ABC transporter has a broader distribution than the MLO, PEN1/SYP121 and ROR2 plasma membrane proteins. It accumulates both in disc-shaped patches at infection sites (Figure 1d) and adjacent to the upper part of developing powdery mildew haustorium [39]. Both of these sites of accumulation appear to coincide with callose deposition in *Arabidopsis* plants that are infected with the barley powdery mildew (*B. graminis hordei*) (S Koh *et al.*, unpublished). Assuming that the PEN3/PDR8 transporter plays a role in the export of toxic molecules, it might act by poisoning both pathogen penetration pegs within the cell-wall space and the haustoria [39].

In addition, non-defense-related plasma membrane proteins accumulate in discrete rings of bright fluorescence, roughly the diameter of papillae, that encircle powdery mildew entry sites (Figure 1c; [10•]). These rings crossed cell boundaries when the penetration site was near the edge of a cell, suggesting that the mechanism that sets up this pattern of protein accumulation in the plasma membrane can operate across cell boundaries.

These observations raise additional questions about processes that underlie this focal accumulation of defense components at pathogen entry points. What are the signals that elicit this focal accumulation? The non-specific elicitor derived from bacterial flagella, flg22, elicits the phosphorylation of the PEN3/PDR8 ABC transporter [43], and also stimulates an increase in *PEN3/PDR8* transcript levels [39]. The role of this phosphorylation step in the functioning and localization of PEN3/PDR8 is unknown. Do other non-specific elicitors or pathogen-associated molecular patterns (PAMPs), such chito-oligomers, ergosterol, or pectin fragments, participate in signaling these subcellular responses? Does the signal diffuse from a point source at the center of the disc to stimulate the disc-like accumulation of proteins? What pattern of signal(s) distribution or what process elicits the multi-ringed bull's eye accumulation of proteins at penetration sites (see Figure 2i of [10•])? Does the focal accumulation depend on new protein synthesis or is it the consequence of redistribution of existing protein? What are the molecular mechanisms that assist in the focal accumulation of such proteins? Work by Bhat *et al.* [23••] indicates that the actin microfilament network does not have a role in the targeted accumulation of MLO and ROR2 or in the maintenance of this focal accumulation. Overexpression of an actin depolymerizing factor did not

block the focal accumulation of MLO or ROR2 at attempted penetration sites in barley cells. What are the molecular mechanisms that sustain the targeted accumulation of these proteins at attempted penetration sites? FRAP experiments suggest that the MLO microdomains are relatively stable once formed; new protein does not enter the microdomain after photobleaching [23••].

Focal accumulation of organelles at penetration sites

The redistribution of plant organelles and the actin cytoskeleton upon pathogen attack has been documented in compatible, incompatible and inappropriate interactions [8,10•,12•,44,45•]. In *Arabidopsis* powdery mildew (*E. cichoracearum*)-infected susceptible *Arabidopsis* cells, the host cytoplasm and all major organelles migrated to penetration sites prior to pathogen entry into host cells (Figure 1e and f; [10•]). Takemoto *et al.* [8] showed that the ER and Golgi bodies accumulated preferentially at attempted penetration sites in both compatible and incompatible interactions between *Arabidopsis* and oomycete pathogens. The accumulation of cytoplasm and organelles occurred in adjacent cells next to penetration sites, suggesting that neighboring cells are in communication (Figure 1f). Koh *et al.* [10•] demonstrated that peroxisomes accumulate to a greater density than the cytoplasm, suggesting that this organelle is preferentially targeted to infection sites. Furthermore, this accumulation of peroxisomes, once completed, was stable in the presence of the actin cytoskeleton inhibitor Latrunculin B (Figure 1f; S Koh *et al.*, unpublished). The association of the PEN2 glycosyl hydrolase with the peroxisomes suggests that this reorganization contributes to inducible forms of penetration resistance [38]. Whether the redistribution of some organelles in compatible interactions facilitates the export of nutrients to the pathogen is unknown. Vesicles that deliver toxic secondary metabolites, pathogenesis-related proteins or cell wall materials to penetration sites are also an important component of the plant's response to pathogens [7,37]. At present, however, few markers exist for these vesicles and our knowledge of their redistribution in time and space relative to pathogen infection is limited. (Figure 1b from [42] shows the focal accumulation of vesicles labeled with YFP-tagged VAMP722.)

As mentioned above, Takemoto *et al.* [8] found that large actin bundles radiate from penetration sites in cells in both compatible and incompatible interactions during the penetration phase of the infection but do not persist. In barley, the overexpression of RACB, a RHO-like small monomeric G-protein family member, reduced the extent of actin cytoskeleton reorganization in response to powdery mildew attack and enhanced susceptibility [44]. Finally, inhibitor studies suggested that the actin microfilament network is required for the plant to mount an effective defense [8,12•,34,45•]. Presumably, the actin cytoskeleton plays an important role in the targeted redistribution of organelles and vesicles to sites of attempted penetration [36].

The extrahaustorial membrane, the plant membrane that encases fungal haustoria, provides the last barrier to pathogens that draw water and nutrients from plant cells via haustoria. Although this membrane appears continuous with the plasma membrane in transmission electron micrographs, studies using biochemical markers and monoclonal antibodies suggest that the extrahaustorial membrane has unique characteristics [46]. Koh *et al.* [10•] showed that eight GFP-tagged plasma membrane proteins are not present in the extrahaustorial membrane, both confirming the distinct nature of this membrane and suggesting that the diffusion of proteins into this membrane is restricted (Figure 1b). The nature of the extrahaustorial membrane and the mechanism by which it is formed remain unknown but represent important challenges for efforts to understand disease development in plants that are infected with haustorium-forming pathogens.

Conclusions

Live-cell imaging of the infection court in plant–pathogen interactions offers new insights into our understanding of plant responses to attack at the single-cell level. Plants have the ability to target responses to subcellular regions where the pathogen attempts to enter the cell. These responses have been best documented at sites of fungal and oomycete invasion, but transmission electron micrographs of bacterial infection sites suggest that subcellular responses might also be targeted to those regions of the cell in direct contact with bacteria [47]. Many questions remain unanswered about the underlying processes that are required for the targeted redistribution of organelles and plasma membrane proteins. The integration of knowledge from mutational studies with information provided by advanced CLSM techniques (using fluorescent tags for specific proteins and organelles) will help us understand how plant defenses are integrated in time and space in attacked cells.

Note added in proof

Recently, Robatzek *et al.* [48] demonstrated that within 20 minutes following treatment with flg22, the flg22 receptor kinase (FLS2–GFP) moved from the plasma membrane to discrete intracellular vesicles. The authors proposed that ligand-stimulated endocytosis provides a mechanism for attenuating FLS2 signaling.

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- • of special interest
- •• of outstanding interest

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Real-time monitoring of the penetration by arbuscular mycorrhizal (AM) fungi into *Medicago truncatula* root epidermal cells revealed the formation of a transient structure, the pre-penetration apparatus (PPA), in this symbiotic interaction. PPA formation included extensive actin microfilament reorganization and was preceded by host nuclear movement to a site just below the fungal penetration point. Supplemental materials for this paper include movies that show hyphal growth through the epidermal cell, following the path of the PPA.

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The authors used live-cell imaging to document the sequence of subcellular responses to the *Arabidopsis* powdery mildew (*E. cichoraceaerum*) over the first 24 hours of the infection sequence. All major host organelles, as well as cytoplasm, accumulated under fungal appressoria prior to the initiation of haustoria formation. At least one type of organelle, the peroxisomes, accumulated to high levels, suggesting that this organelle was not simply carried passively to the penetration site by the cytoplasm. The use of GFP-tagged plasma membrane marker proteins revealed the elaboration of membrane pouches prior to the maturation of haustoria. The necks of mature haustoria, however, were encircled by only a small collar of plasma membrane. The plasma membrane markers appeared to be excluded from the extrahaustorial membrane encasing the haustorium.

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