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Interconnection between actin cytoskeleton and plant defense signaling

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Actin cytoskeleton is the fundamental structural component of eukaryotic cells. It has a role in numerous elementary cellular processes such as reproduction, development and also in response to abiotic and biotic stimuli. Remarkably, the role of actin cytoskeleton in plant response to pathogens is getting to be under magnifying glass. Based on microscopic studies, most of the data showed, that actin plays an important role in formation of physiological barrier in the site of infection. Actin dynamics is involved in the transport of antimicrobial compounds and cell wall fortifying components (e.g. callose) to the site of infection. Also the role in PTI (pathogen triggered immunity) and ETI (effector triggered immunity) was recently indicated. On the other hand much less is known about the transcriptome reprogramming upon changes in actin dynamics. Our recently published results showed that drugs inhibiting actin polymerization (latrunculin B, cytochalasin E) cause the induction of genes which are involved in salicylic acid (SA) signaling pathway. In this addendum we would like to highlight in more details current state of knowledge concerning the involvement of actin dynamics in plant defense signaling.

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During their life, plants are as all other living organisms, constantly exposed to changing environmental conditions including pathogen attack. In comparison with other organisms, plants lack the possibility to escape from their enemies. Even so our planet is still "green" and surrender of plant to pathogen is rather exception than the rule. This fact indicates that the very successful complex plant defense mechanisms exist (e.g., PTI and ETI), for review see.[1,2] It has recently been shown that very important platform for plant defense against pathogens comprises actin cytoskeleton and its dynamics.[3,4] Nowadays the role of actin cytoskeleton in defense against fungi and oomycetes is studied more frequently. In these responses actin serves as a tool for physical barrier formation, as a key player in vesicular trafficking responsible for transport of callose, antimicrobial compounds and cell wall components to the site of infection.[3] Outbreaking work of Tian et al. (2009) described that actin dynamics is involved also in the defense against pathogenic bacteria.[5] Although since that time new findings are arising, our current knowledge about mechanism how actin dynamics regulates and enhances plant defense is still not fully understood.

Proper Function of Actin Cytoskeleton Inhibits Pathogen Penetration

The physical barriers (e.g. cuticle, trichomes) represent the first problem for pathogen successful invasion. Stomata are weak point of this kind of defense, but it was shown that actin plays a role in stomata closing.[3] Treatment with cytochalasin D causes the opening of stomata,[6] on the other hand treatment with latrunculin B increases the level of abscisic acid which induces stomatal closure.[7] These "contradictory" results can be explained by the different inhibitory mechanism of actin polymerization caused by these drugs.[8-10] Higaki et al. (2010) showed that spatial arrangement of the filaments (not only bundling and organization) influences guard cells dynamics.[11] While

the actin filaments bundle on the site of penetration, the disruption of actin either by actin depolymerizing drugs or by overexpression of ADF4 (actin depolymerizing factor 4) eases penetration of pathogens into plant tissue. In addition, for callose deposition in the cell walls vesicular trafficking is indispensable process in which actin cytoskeleton plays an important role.[3-5,12-22] Based on these findings, actin dynamics is crucial for both fine tuning of host and non-host resistance. Especially in defense against fungi and oomycetes actin dynamics represents one of the key component in formation of physical barrier against their penetration.

Role of Actin Cytoskeleton in PTI and ETI

Since the Tian et al. (2009) published their work, intriguing question how actin dynamics is involved in PTI and/or ETI aroused.[5] PTI is ancient defense reaction base on the recognition of pathogen-associated molecular patterns (PAMPs; e.g., flg22, elf26) whereas ETI is host reaction based on the effectors secreted (e.g., AvrPphB) by pathogen. Using pathogenic bacteria *Pseudomonas syringae* pv *tomato* DC3000 AvrPphB and *Arabidopsis thaliana* knock-out mutant *Atadf4* Tian et al. (2009) showed that AvrPphB effector triggers signal transduction through ADF4. Plants deficient in ADF4 are not able to induce ETI in the presence of AvrPphB, which means that *Atadf4* mutants are more susceptible to bacteria carrying AvrPphB.[5]

Subsequently Porter et al. (2012) showed that ADF4 is required for expression of *RPS5* gene which encodes a well-known R-protein. Moreover in *Atadf4* mutant the activation of MPK3 and MPK6 (mitogen activated protein kinase) is inhibited. Both MPKs play an important role in the establishment of PTI and/or ETI.[23] Additionally, without fully running ETI in *Atadf4,* the expression of *FRK1* (flg22-induced receptor-like kinase 1), the early response defense gene to flg22 (conserved epitope of flagellin), is inhibited by AvrPphB.[24] Henty-Ridilla et al. (2013) showed that upon PTI elicitation, actin filament density is increased until 18 hpi compared to control, followed by decrease of its density in later time. This decrease is very probably caused by salicylic acid and subsequent bundling of filaments which starts at 18 hpi and proceeds in later phases.[20,27] The increase of filament density requires functional BIK1 (botrytis-induced kinase 1) and BAK1 (bri1-associated receptor kinase 1), important components of PTI. It supports the idea that actin cytoskeleton is involved in PTI defense. Additionally treatment with latrunculin B promotes bacterial growth.[20] Very recently another publication from Steiger´s lab showed that ADF4 regulates actin dynamics during PTI.[25] They observed that in dark grown Arabidopsis seedlings, the elicitation of PTI by bacterial MAMP (microbe associated molecular pattern) elf26 (conserved epitope of elongation factor Tu) leads to increased density of actin filaments which is caused by higher rate of severing of filaments upon treatment. In *Atadf4* mutant plants, the severance is inhibited but not induced by elf26 treatment and filament density does not increase upon elf26 treatment. This is not the case of elicitation by chitin (fungal MAMP), which also causes the increase of filament density, independently on ADF4.[25] It indicates that actin dynamics in response to different stimuli is regulated by distinct mechanism. Another point of view into the plant-pathogen arms race brought the recognition that *Pseudomonas syringae* effector HopW1, which disrupts the actin cytoskeleton in the same manner as latrunculin B promotes bacterial virulence. HopW1 and also latrunculin B inhibit endocytosis in Arabidopsis.[19] Intriguingly as a consequence recognition of flg22 by FLS2 (flagellin-sensitive two) receptor and further signaling is dependent on endocytosis.[26] Taken together it brings possible explanation how HopW1 (or actin disruption respectively) triggers the suppression of PTI.

Actin Dynamics and Transcriptomic Changes

Kobayashi et al. (2007) showed that in tobacco plants 48h after treatment with cytochalasin E induced *PR-1* (*PATHOGENESIS RELATED 1*) transcription.[28] Recently we showed that the inhibition of actin polymerization by latrunculin B and cytochalasin E leads to the induction of SA related genes (including *PR-1*) in Arabidopsis seedlings.[27] Interestingly, *Atadf4* mutant was delayed in the expression of *PR-1 g*ene upon pathogen treatment.[5] It supports the possibility that actin dynamics is involved in *PR* genes transcription. Most publications in which treatment with latrunculin B or cytochalasin E together with bacteria was used, showed higher susceptibility of plants to bacteria.[20] At the first glance these findings are contradictory to our results that latrunculin B induces transcription of defense genes.[27] But in the light of current knowledge these 2 effects are probably not connected as the timing of events plays an important role. Probably the inhibition of actin cytoskeleton dynamics (structural properties) and inhibition of PTI response (due to inhibition of endocytosis) leads to higher susceptibility of host plant to pathogen. On the other hand evidence that depolymerisation of actin cytoskeleton is important for ETI,[5] enables us to hypothesize that changes in actin dynamics caused by latrunculin B lead to the activation of ETI or the process which we can call ETI-like response (hypersensitive reaction, MPKs activation) and resulting in the induction of *PR* gene transcription. In fact this hypothesis has to be further confirmed since our understanding of the effect of actin dynamics on transcriptomic changes is still insufficient. The mechanism how actin dynamics is involved in PTI and ETI seems to be very inspiring field for further investigations which can lead to fascinating observations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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