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Plant immunity: the EDS1 regulatory node

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ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and its interacting partner, PHYTOALEXIN DEFICIENT 4 (PAD4), constitute a regulatory hub that is essential for basal resistance to invasive biotrophic and hemi-biotrophic pathogens. EDS1 and PAD4 are also recruited by Toll-Interleukin-1 receptor (TIR)-type nucleotide binding-leucine rich repeat (NB-LRR) proteins to signal isolate-specific pathogen recognition. Recent work points to a fundamental role of EDS1 and PAD4 in transducing redox signals in response to certain biotic and abiotic stresses. These intracellular proteins are important activators of salicylic acid (SA) signaling and also mediate antagonism between the jasmonic acid (JA) and ethylene (ET) defense response pathways. EDS1 forms several molecularly and spatially distinct complexes with PAD4 and a newly discovered *in vivo* signaling partner, SENESCENCE ASSOCIATED GENE 101 (SAG101). Together, EDS1, PAD4 and SAG101 provide a major barrier to infection by both host-adapted and non-host pathogens.

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Current Opinion in Plant Biology 2005, **8**:383–389

This review comes from a themed issue on
Biotic interactions
Edited by Paul Schulze-Lefert and Edward Farmer

Available online 6th June 2005

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2005.05.010

Introduction

Individual plant cells perceive an enormous range of external cues. Plant survival depends on integrating this information and responding appropriately in terms of metabolism, growth and defense. In the natural environment (and indeed the cleanest growth chamber!) plants are rarely able to grow without attempted pathogen colonization and have evolved an elaborate, multi-layered system of innate immunity. Unraveling these layers and comprehending how the most aggressive pathogens overcome or subvert defenses to cause disease is of major interest. Some of the most effective barriers to disease are expressed at the plant cell wall and plasma membrane, preventing pathogen penetration and accounting for the majority of aborted infections in non-host (species-level) resistance. Necrotrophs commonly take advantage of wound sites or dead cells to invade. By contrast, biotrophic and hemi-biotrophic pathogens have evolved specialized structures and effector molecules that allow invasive growth on particular host genotypes and, in the case of obligate biotrophs, limit the disruption of host cell integrity.

The contrasting modes of infection of necrotrophs at one extreme and obligate biotrophs at the other require ingenuity in plant defense signaling. What emerges from genetic analyses, mainly of *Arabidopsis*, is a complex circuitry that balances the activation of various basal defenses. Pathways involving the hormones jasmonic acid (JA), JA-related oxygenated lipids and ethylene (ET) are principally effective against necrotrophic pathogens and chewing insects, whereas those involving salicylic acid (SA) are effective against biotrophs [1]. The expression of basal resistance to invasive pathogens is a crucial protective layer. Without it, plants become super-susceptible to even mild infections and are less likely to survive in a competitive environment. A large catalogue of *Arabidopsis* mutants that are compromised in basal defenses to virulent pathogens points to the involvement of many genes in maintaining this resistance layer and to the existence of numerous potential targets that the pathogen might disable to promote disease [2]. A further layer of resistance to invading pathogens is mediated by *Resistance* (*R*) genes that encode proteins that recognize the presence of specific pathogen effector molecules. Recognition triggers dramatic cellular reprogramming that stops pathogen growth, and often involves a localized burst of reactive oxygen intermediates (ROI) and strictly delimited programmed plant cell death. The local response also serves to prime uninfected tissues against subsequent attack in a process called systemic acquired resistance [3•]. Several key plant defense regulators have been cloned and characterized. In this review, we discuss ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1), a positive regulator of basal resistance to invasive biotrophs and hemi-biotrophs that is also indispensable for Toll-Interleukin-1 receptor (TIR)-type nucleotide binding-leucine rich repeat (NB-LRR) protein-triggered resistance. We highlight several recent studies that suggest that EDS1 and its partners are positioned as a pivotal node in signal relay against pathogens and in certain abiotic stress responses.

Positioning EDS1 and its partner PAD4 in the defense signaling network

EDS1 was originally identified in a screen for mutants that are defective in *RPP1*- and *RPP5*-specified resistance to

isolates of the obligate biotrophic oomycete pathogen *Peronospora parasitica* [4]. Further inspection of *eds1* mutants revealed defects in basal resistance to virulent isolates of *P. parasitica* and *Erysiphe* (an obligate biotrophic fungus) and to strains of the bacterial pathogen *Pseudomonas syringae* (notably *P. syringae tomato* [Pst] DC3000 and *P. syringae maculicola* [Psm] ES4326], which cause bacterial leaf spot) [4–6]. *PAD4*, which encodes a protein that interacts with EDS1 *in vivo* [7], was first discovered among several mutants in a cleverly conceived screen for enhanced disease susceptibility to low doses of virulent Psm [8]. Additional *pad4* mutant alleles emerged from the *RPP* resistance screens [7].

EDS1 and *PAD4* were cloned in 1999 and both found to have pockets of homology to eukaryotic lipases [9,10]. Pathology assays revealed that they were required genetically by the same spectrum of *Arabidopsis R* genes that belong to the intracellular TIR-NB-LRR class, consistent with the notion of *EDS1–PAD4* cooperation in defense signaling [5,7]. NB-LRR genes that possesses an amino-terminal coiled-coil (CC) domain rather than a TIR domain triggered resistance independently of *EDS1* and *PAD4*, suggesting that these defense regulators might constitute a point of signal discrimination between the two classes of intracellular immune receptor [5]. Absolute discrimination is an over simplification, although the recruitment of *EDS1* in TIR-NB-LRR-conditioned resistance is conserved in other plant species [11,12,13•]. Close inspection of *eds1* and *pad4* null mutant phenotypes in *Arabidopsis* showed that *EDS1* exerts an early activity in TIR-NB-LRR resistance, acting upstream of the oxidative burst and programmed cell death. EDS1 and PAD4, together, are required for SA accumulation and for defense potentiation involving the processing of ROI-derived signals around infection foci [7,14]. SA itself contributes to the expression of both *EDS1* and *PAD4* as part of a positive feedback loop that appears to be important in defense amplification [9,10,15,16,17•,18•]. The essentially equivalent activities of *EDS1* and *PAD4* in basal resistance and defense signal potentiation were separable from *EDS1*-dependent *TIR-NB-LRR* gene-triggered ROI generation and localized programmed cell death, implying that EDS1 has an additional activity in the R-protein-triggered cascade. From an evolutionary perspective, the involvement of *EDS1* and *PAD4* in basal resistance is likely to reflect their ancestral functions because rice and the other monocotyledonous species tested to date lack TIR-NB-LRR *R* genes but express orthologs of *EDS1* and *PAD4* ([19]; http://www.tigr.org/tdb/e2k1/osa1/ and http://barley.ipk-gatersleben.de/ebdb.php3). Can we position EDS1 and PAD4 accurately in TIR-NB-LRR mediated defense? EDS1 and PAD4 activities that are coincident or immediately downstream of R-protein activation are supported by evidence that these components are required in constitutive resistance triggered by several auto-activated variants of TIR-type NB-LLR proteins [16,20,21••]. If deregulated R proteins feed signals into a potentiating loop, however, the start and finish of the cycle become difficult to de-merge.

Several *Arabidopsis* genes (identified in mutational screens) that negatively regulate the EDS1 pathway provide additional clues to the position of EDS1 and PAD4 in the defense signaling network [14,22,23,24•]. An important question is how directly these genes impact on EDS1 and PAD4. *LESIONS SIMULTATING DISEASE 1* (*LSD1*) which encodes a zinc-finger protein, behaves as an ROI modulator and holds an EDS1/PAD4-dependent cell-death pathway in check [24•]. Also, *MAP kinase 4* (*MPK4*) negatively regulates SA accumulation and related systemic defenses through EDS1 and PAD4 but promotes induction of the JA pathway [22,24•]. Thus, MPK4 appears to constitute a node in the inhibitory cross-talk between the SA and JA signaling networks. Follow-up studies by Mundy and colleagues reveal that MPK4 stimulates JA and ET signaling in resistance to the necrotrophic pathogen *Alternaria brassicicola* (P Brodersen, J Mundy, personal communication). Significantly, the JA- or ET-activating functions of MPK4 are repressed by EDS1 and, to a lesser extent, by PAD4. The results show that EDS1 and PAD4 are involved in controlling signal antagonism between SA and JA/ET defenses, as was hinted at in earlier studies [25,26]. An entirely different gene, *ACCELERATED-CELL-DEATH11* (*ACD11*) encodes a protein that has *in vitro* sphingosine transfer activity and represses a programmed cell-death pathway that again relies on EDS1 and PAD4 [23]. These findings point to the possible impact of sphingolipids on EDS1 signaling, although it is not known whether ACD11's sphingolipid-binding activity is involved in repressing the EDS1/PAD4-dependent cell-death pathway.

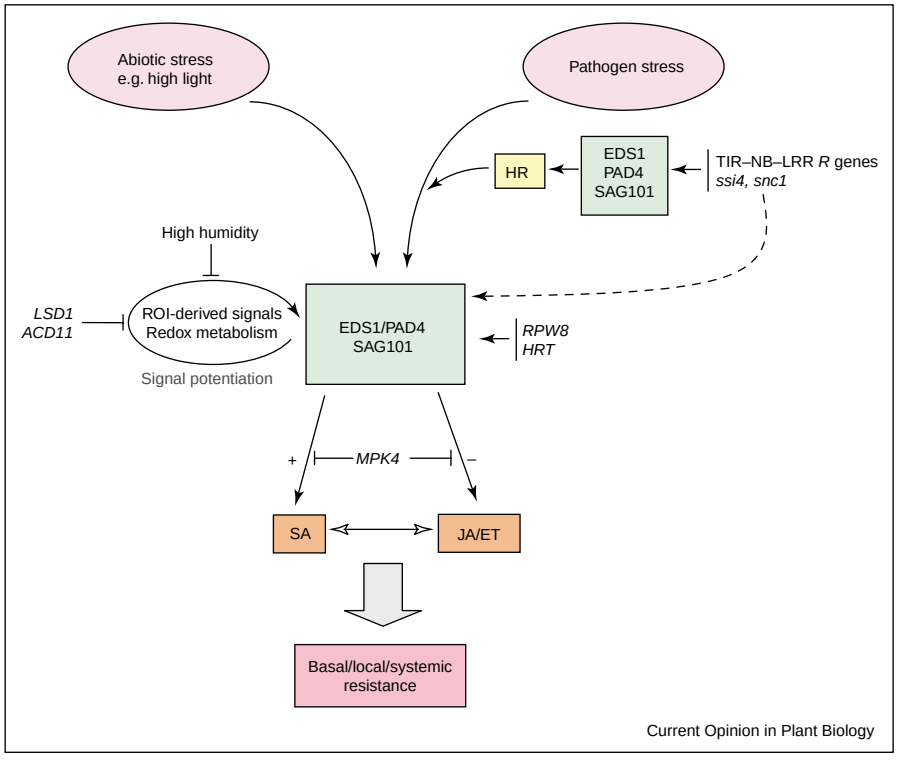
EDS1 and redox stress signal relay

Accumulation of evidence of more fundamental activities of EDS1 and PAD4 in transducing redox signals has gathered some momentum. Rustérucci *et al.* [14] revealed the existence of an ROI- and SA-stimulated propagative loop that requires EDS1 and PAD4 in *lsd1*-conditioned runaway cell death. Further work by Karpinski and colleagues [24•] shows that *lsd1* mutants fail to acclimate to excess excitation energy (EEE) generated by photosynthesis in high light, causing ROI overload and ultimately cell death due to photooxidative stress. Normally EEE is dissipated by a combination of photorespiratory and antioxidant systems. *lsd1* mutants exhibit several defects, including reduced stomatal conductance and reduced peroxisomal catalase activity that both lead to increased ROI. Application of SA itself reduced stomatal conductance and, as a known inhibitor of antioxidant enzymes [3•], would further exacerbate redox imbalance. Importantly, EDS1 and PAD4 were necessary components in all of the *lsd1* photooxidative-stress phenotypes, including stomatal closure [24•] A unifying feature of EDS1 and

PAD4 activities in pathogen resistance and certain abiotic stress responses might therefore be in processing, directly or indirectly, ROI-derived molecules (Figure 1).

Both redox and sphingolipid metabolites have been shown to be important for the function of stomatal guard cells [27]. The suppressive effects of high humidity (described as a 'humidity-sensitive factor') on EDS1–PAD4-dependent pathogen resistance and cell death conditioned by the constitutively active TIR-NB-LRR protein ssi4 [28] or by the CC membrane-associated powdery mildew resistance components RPW8.1 and RPW8.2 [6,17•] might be rationalized in the context of EDS1-mediated ROI signal relay. Similarly, constitutive *EDS1–PAD4*-dependent pathogen resistance and growth defects in the *bonzai1* (*bon*; also called *copine1* [*cpn1*]) mutant are suppressed by high humidity [29]. *BON1* encodes a Ca^{2+}-dependent phospholipid-binding protein that negatively regulates *SUPPRESSOR OF NPR1-1, CONSTITUTIVE 1* (*SNC1*), a member of the *RPP4 TIR-NB-LRR* locus [21••]. It may also be significant that the increased drought tolerance resulting from an activation-tagged allele of a *CC-NB-LRR*-type gene, *ADR1*, depends on *EDS1* [30]. The mechanistic details remain to be worked through, but the importance of redox metabolism in the responses described above prompts further definition of intracellular and apoplastic redox systems and characterization of ROI-generated signals and consequent protein modifications. It is now known that translocation from the cytosol to the nucleus of NON EXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1), an SA response regulator, and its

Figure 1



Integration of biotic and abiotic stress responses through the EDS1 family of resistance regulators. *Arabidopsis* EDS1, PAD4 and a newly identified *in vivo* EDS1-interactor, SAG101 (M Wiermer, B Feys and J Parker unpublished), constitute a regulatory node that couples R-protein-mediated pathogen recognition to activation of basal defenses. In TIR-NB-LRR-protein-triggered responses, EDS1 and its partners are needed for expression of hypersensitive plant cell death (HR) and accumulation of salicylic acid (SA). Another function of these regulators is in generation of a signal potentiation loop that involves processing of ROI- and SA-derived signals. Cellular homeostasis of ROI molecules generated during photooxidative stress, and normally dissipated in part through the action of the zinc finger protein LSD1, is controlled by EDS1 and PAD4. Both LSD1 and ACD11 (a sphingosine transfer protein) are negative regulators of a cell death pathway that depends on EDS1 and PAD4 activities. Environmental conditions, such as high humidity, can abrogate certain EDS1/PAD4-dependent responses, including *lsd1*-controlled cell death and *ssi4*-triggered resistance. Deregulated R proteins, such as ssi4 and snc1 of the TIR-NB-LRR class, may connect to the EDS1 pathway at two possible entry points: upstream of the HR and/or into the signal potentiation loop. Certain non-TIR-NB-LRR type R proteins such as RPW8 and HRT have been shown to require EDS1 and PAD4 for full resistance, possibly reflecting their requirement for SA-pathway amplification. The MAP kinase MPK4 constitutes an important control point that negatively regulates both the positive and antagonistic effects of EDS1 and PAD4, respectively, on SA- and jasmonic acid/ethylene (ET/JA)-mediated defense. SAG101 was shown to have partially overlapping functions with PAD4 in basal and TIR-NB-LRR mediated resistance. Its involvement in other aspects of defense/stress signaling depicted in this figure has not yet been demonstrated.

interaction with certain TGA transcription factors in the nucleus to drive defense gene expression, is under tight redox control [3•]. Other *Arabidopsis* genetic components that link ROI molecules to downstream stress responses have recently been uncovered [31,32].

Additional players in EDS1 defense relay

Arabidopsis EDS1 and PAD4 interact in soluble cell extracts of healthy (pathogen unchallenged) leaves, indicating the presence of a pre-existing EDS1–PAD4 complex, although the co-immunoprecipitable amounts of EDS1 and PAD4 increased upon pathogen challenge [7]. The molecular interactions and biochemical activities of EDS1 and PAD4 need to be defined more precisely. Using an affinity-purification approach coupled to Quadropole-Time-of-flight (Q-TOF) mass spectrometry, an additional EDS1 interactor, SAG101, has been identified in healthy leaf extracts from a tagged EDS1 transgenic line (B Feys, M Wiermer and J Parker, unpublished). This is interesting, first because SAG101 had not emerged as an EDS1 interactor in yeast two-hybrid screens. Second, *Arabidopsis* (and other plant) SAG101 proteins share a conserved (EP) domain in their carboxy-terminal halves with EDS1 and PAD4. SAG101 also has some lipase homology but unlike EDS1 and PAD4 does not possess amino acids that constitute a putative serine-hydrolase catalytic triad (B Feys, M Wiermer and J Parker, unpublished). A form of SAG101 was identified previously as a positive regulator of senescence and was reported to have acyl hydrolase activity *in vitro* [33]. Analysis of T-DNA-insertion mutants of *SAG101* alone or of *SAG101* in combination with *pad4* revealed that *SAG101* possesses a defense regulatory function that is partially redundant with *PAD4* in both TIR-NB-LRR-type *R*-gene-mediated resistance and basal resistance (B Feys, M Wiermer and J Parker, unpublished; see also Figure 1). The PAD4 and SAG101 proteins failed to accumulate in an *eds1* background, suggesting that EDS1 might act as a kind of scaffold for PAD4 or SAG101 activities. Several molecularly distinct EDS1 complexes could be distinguished in the cytosol and nucleus, providing a possible framework for the trafficking of signals between cellular compartments. Notably, a predicted nucleoporin 96 that localizes to the nuclear envelope was recently identified as an additional component of *R*-gene-mediated and basal resistance [34•].

Pathology phenotyping of *pad4|sag101* double mutants revealed defects in TIR-NB-LRR *R*-gene-mediated resistance to avirulent pathogens and in basal resistance to virulent pathogens that were equivalent to or even more extreme than the phenotypes of *eds1* mutants (B Feys, M Wiermer and J Parker, unpublished). In another study, the Schulze-Lefert group [35] attempted to genetically 'peel' the layers of non-host resistance to isolates of powdery mildew that normally infect barley (*Blumeria graminis* f. sp. *hordeii*) or pea (*Erysiphe pisi*). These isolates largely fail to penetrate *Arabidopsis* epidermal cells unless surface resistance is disabled (J Dittgen, V Lipka and P Schultze-Lefert, personal communication). In wildtype *Arabidopsis*, occasional spore germlings breach the surface layer but these rapidly induce epidermal cell death and grow no further [35]. The *pad4|sag101* double mutant (significantly more so than *eds1*) was found to permit invasive growth of the non-host powdery mildew isolates that was sufficient to enable pathogen sporulation (J Dittgen, V Lipka and P Schultze-Lefert, personal communication). Therefore, the combined activities of PAD4 and SAG101 constitute a major basal resistance layer to both host and non-host pathogens. These new findings add to those of previous studies that establish both common underlying processes and distinctions between host and non-host resistance responses involving the EDS1 pathway [4,36•,37•].

The lipid link

Various studies have shown that EDS1 and PAD4 do more than simply regulate SA in R-protein-triggered and basal resistance [7,24•,38,39], but the nature of the signals that they transduce and their precise biochemical activities remain unclear. Although EDS1 and PAD4 (and less convincingly, SAG101) have homology to acyl hydrolases, no enzymatic activity has been measured to date for any of these proteins in our assays (S Rietz, J Parker, unpublished). Still, the idea that they could process an oxygenated lipid that is produced enzymatically or non-enzymatically upon pathogen infection is rather persuasive [40].

An increasing body of evidence points to the action of lipid metabolites, besides jasmonates and related oxygenated lipids, as important regulators of local and systemic defenses and of cross-talk between the SA and JA/ET pathways [40,41]. Several specific findings are worth highlighting. First, SUPPRESSOR OF FATTY ACID DESATURASE 1 (SFD1), a dihydroxyacetone phosphate reductase that is involved in glycerolipid metabolism [42•], and DEFECTIVE IN INDUCED RESISTANCE1 (DIR1), a putative lipid transfer protein [43], contribute to the establishment of systemic resistance. *EDS1* and *PAD4* are also necessary for the establishment of SAR (L Jorda, A Maldonado, J Parker, C Lamb, unpublished). This defect, coupled with a failure of *eds1* and *pad4* mutants in both signal emission and distal signal perception (L Jorda, A Maldonado, J Parker, C Lamb, unpublished), is consistent with known roles of EDS1 and PAD4 as defense potentiators [14]. It remains to be established whether DIR1 is a systemic component of an EDS1–PAD4-driven amplification system, although preliminary data suggest that DIR1 localizes to the vasculature (phloem and xylem parenchyma) and might therefore be involved in the transport of a lipid signal to systemic tissues (R Cameron, pers. comm.). Second, a protein that has high SA-binding affinity, SALICYLIC

ACID-BINDING PROTEIN 2 (SABP2), has been purified from tobacco and found, by virus-induced gene silencing, to be necessary for the full expression of basal and systemic resistance to tobacco mosaic virus infection [44•]. Crystal structural and biochemical analyses reveal that SABP2 has acyl hydrolase activity, with methyl salicylate as a substrate and SA as product inhibitor [45••]. Together, these findings suggest that lipase and/or lipid-binding activities impact at multiple levels of plant immunity and are worth further biochemical characterization and profiling as candidate lipid signals.

Conclusions

The emerging importance of EDS1 as a central regulatory protein in biotic and oxidative stress signaling (Figure 1) prompts us to explore the structures, interaction dynamics and biochemical activities of EDS1 and its partners in more depth. Although the lipase homologies might be a scientific 'falsche Fährte' ('red herring') in terms of catalytic activity, conservation of these domains in all of the plant EDS1 and PAD4 orthologs examined suggests they are needed, perhaps as structural rather than as enzymatic motifs. We cannot entirely exclude the possibility that EDS1 and its affiliates passage rather than hydrolyze oxygenated lipids inside the cell.

Acknowledgements

We thank all colleagues who provided unpublished data for this review. Work in the Parker laboratory is funded by The Max-Planck Society, The Alexander von Humboldt Foundation and grants from The Deutsche Forschungsgemeinschaft and The Bundesministerium für Bildung und Forschung. We thank our colleagues Steffen Rietz and Stefan Malonek for stimulating discussion while writing this review.

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Arabidopsis BON1 is a member of the highly conserved copine gene family. It encodes a plasma-membrane-associated protein that is required for growth homeostasis and that binds phospholipids in a Ca^{2+}-dependent manner *in vitro*. The authors of this paper describe a further significant finding that the *bon1* mutant phenotype of retarded growth at 22 °C depends on the presence of the *TIR-NB-LRR* gene *SNC1*, which conditions *Peronospora* resistance. Thus, BON1 negatively regulates SNC1 and is a potential virulence target in *Peronospora* infection that might be 'guarded' by SNC1. It is interesting that the transcriptional upregulation of *SNC1* in the *bon1* mutant through an *EDS1/PAD4*- and SA-dependent amplification loop is subject to temperature control (see also [29]). At 28 °C, *SNC1*, *EDS1* and *PAD4* transcript levels are dramatically reduced, thereby attenuating the growth defects and enhanced resistance of *bon1* at high temperature.

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The authors introduce an exciting new dimension to the study of plant defense regulators by analyzing the crystal structure of SABP2 alone and in complex with SA. They show that SABP2 uses methyl-salicylate as a substrate and that SA acts as a potent product inhibitor. The kinetics of SABP2 offer a potential mechanism for the fine-tuning of SA-dependent resistance responses.