Signaling and Communication in Plants

František Baluška *Editor*

Long-Distance Systemic Signaling and Communication in Plants

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Preface

Higher plants coordinate and integrate their tissues and organs via diverse long-distance signalling and communication circuits. Sophisticated sensory systems sensitively screen both internal and external factors and feed sensory information into chemical and physical systemic long-distance communication cascades. Obviously, our view of plants is changing dramatically. We realize that their long-distance signalling is fast, and signals, both of endogenous and exogenous origin, spread rapidly throughout their bodies. This recent revolution in our understanding of higher plants started more than 40 years ago with the discovery of alarm peptide hormone systemin (Green and Ryan 1972; Ryan and Pearce 2003) and continues with rapid advances further. This volume of the 'Signalling and Communication in Plants' series captures the current dynamic 'state of the art' of this very exciting topic of plant sciences.

In general, there are chemical and physical mechanisms for the long-distance signalling and communication in plants. With respect to chemical communication, the most advanced topics are systemic acquired resistance (SAR), which is an inducible defence syndrome based on salicyl acid signalling (Ross 1966; Sticher et al. 1997; Chaturvedi et al. 2012; Wu et al. 2012), and systemic acquired acclimation (SAA), which is systemic signalling of photo-oxidative stress (Karpinski et al. 1999; Karpinski and Szechynska-Hebda 2010). Both SAR and SAA include several aspects of plant memory and anticipation of future insults via the memorized sensory perceptions, using quantum computing including quantum-redox sensing (Szechynska-Hebda et al. 2010; Karpinski and Szechynska-Hebda 2010). Importantly in this respect, both SAR and SAA are based on ROS and hormonal signalling pathways, but also include very rapid electrical and mechanical long-distance signalling. Another extensively investigated and well-understood topic is the long-distance wound signalling based on the alarm peptide hormone systemin and oxylipin-derived jasmonic acid (Farmer and Ryan 1990; Ryan and Pearce 2003, Sun et al. 2011). The next long-distance system is induced systemic resistance (ISR), which is induced by diverse non-pathogenic agents such as growth-promoting rhizobacteria and other plant beneficial microorganisms (van Wees et al. 2000; Rudrappa et al. 2010; Berendsen et al. 2012; Lee et al. 2012).

The nature of root-to-shoot long-distance communication is still not well understood for the ISR, but besides salicyl acid and jasmonic acid, abscisic acid is also involved (Kumar et al. 2012; Sampath Kumar and Bais 2012). Root-to-shoot long distance is also involved in the initiation and control of the symbiotic Rhizobia bacteria interactions with legume roots via so-called social media pathway (Venkateshwaran et al. 2013). Interestingly, this 'social media' pathway is also supporting long-distance interactions between roots and arbuscular mycorrhizal fungi (Venkateshwaran et al. 2013), which help plants to acquire nutrients, especially phosphate, and solutes. Last but not least, phosphate and iron homeostasis in plants is also safeguarded via long-distance signalling pathways and circuits (Enomoto et al. 2007; Enomoto and Goto 2008; Nagarajan et al. 2011; Smith et al. 2011).

Physical mechanisms of long-distance signalling and communication in plants include both electrical and mechanical/hydraulic mechanisms. In fact, electrical signals were discovered in plants more than 140 years ago (Burdon-Sanderson 1873, 1899; Stahlberg 2006). Although the plant action potentials show the same bioelectric parameters like animal/humans action potentials, they are driven by slightly different ion channels and other molecules (Fromm and Lautner 2007; Hedrich 2012; Baluška and Mancuso 2013). Despite this long tradition in plant electrophysiology, the importance and roles of plant action potentials for plant physiology and plant behaviour are still rudimentary (Brenner et al. 2006). However, it emerges that electric long-distance signalling in plants is more complex than that in animals because it includes also variation potentials, system potentials, and hydraulic signals (Malone 1992; Stahlberg 2006; Stahlberg et al. 2005; Zimmermann et al. 2009). It is also obvious that root apices and phloem represent the most active sites of electric activity in plants (Masi et al. 2006; Fromm and Bauer 1994; Fromm and Lautner 2007; Baluška and Mancuso 2013).

Another important and relatively well-understood topic in plant long-distance signalling and communication is that of mobile RNA molecules that move within the phloem (Lucas et al. 2001; Banerjee et al. 2006, 2009). Besides coding mRNAs, also non-coding regulatory RNAs are moving within plants (Schwab et al. 2009; Molnar et al. 2011), which is related to systemic propagation of the acquired stress-induced epigenetic changes (Molnar et al. 2011). For example, systemic acquired silencing (SAS) is rather a well-understood phenomenon studied in plants for more than a decade (Palauqui et al. 1997). Phloem elements are really unique as they represent supracellular highways for plant long-distance signalling, spanning throughout the whole plant body—integrating it into functional unity, using all kinds of diverse long-distance signalling and communication pathways (Lucas et al. 2001; Van Bel and Hafke 2013).

The final chapter of this volume is devoted to the emerging topic of long-distance signalling and communication in plants: herbivore-induced volatile organic compounds (VOCs) that act as semiochemical signals, playing roles in both the within-plant and plant–plant communication (Baldwin et al. 2006; Girón-Calva et al. 2012; Rodriguez-Saona et al. 2013). One important aspect of this new and important topic is the ability of VOCs to prime defenses in plants by enhancing

their resistance and responses to subsequent herbivore attacks (Kobayashi et al. 2006; Ton et al. 2007; Verheggen et al. 2010). Importantly, this long-distance signalling and communication via phytosemiochemicals has great potency for improving crop protection and efficiency of agriculture (Bruce 2010; Jansen et al. 2010; Khan et al. 2010).

Bonn, Germany František Baluška

References

Baldwin IT, Halitschke R, Paschold A, von Dahl CC, Preston CA (2006) Volatile signaling in plant-plant interactions: "talking trees" in the genomics era. Science 311:812–815

Baluška F, Mancuso S (2013) Ion channels in plants: from bioelectricity, via signaling, to behavioral actions. Plant Signal Behav 8:1–4

Banerjee AK, Chatterjee M, Yu Y, Suh SG, Miller WA, Hannapel DJ (2006) Dynamics of a mobile RNA of potato involved in a long-distance signaling pathway. Plant Cell 18:3443–3457

Banerjee AK, Lin T, Hannapel DJ (2009) Untranslated regions of a mobile transcript mediate RNA metabolism. Plant Physiol 151:1831–1843

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Brenner ED, Stahlberg R, Mancuso S, Vivanco J, Baluska F, Van Volkenburgh E (2006) Plant neurobiology: an integrated view of plant signaling. Trends Plant Sci 11:413–419

Bruce TJA (2010) Exploiting plant signals in sustainable agriculture. In: Baluška F, Ninkovic V (eds) Plant communication from an ecological perspective. Springer, Berlin, pp 215–227

Burdon-Sanderson J (1873) Note on the electrical phenomena which accompany irritation of the leaf of *Dionaea muscipula*. Proc R Soc Lond 21:495–496

Burdon-Sanderson J (1899) On the relation of motion in animals and plants to the electrical phenomena, which are associated with it. Proc R Soc Lond 65:37–64

Chaturvedi R, Venables B, Petros RA, Nalam V, Li M, Wang X, Takemoto LJ, Shah J (2012) An abietane diterpenoid is a potent activator of systemic acquired resistance. Plant J 71:161–172

Enomoto Y, Goto F (2008) Long-distance signaling of iron deficiency in plants. Plant Signal Behav 3:396–397

Enomoto Y, Hodoshima H, Shimada H, Shoji K, Yoshihara T, Goto F (2007) Long-distance signals positively regulate the expression of iron uptake genes in tobacco roots. Planta 227:81–89

Farmer EE, Ryan CA (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc Natl Acad Sci USA 87:7713–7716

Fromm J, Bauer T (1994) Action potentials in maize sieve tubes change phloem translocation. J Exp Bot 273:463–469

Fromm J, Lautner S (2007) Electrical signals and their physiological significance in plants. Plant Cell Environ 30:249–257

Girón-Calva PS, Molina-Torres J, Heil M (2012) Volatile dose and exposure time impact perception in neighboring plants. J Chem Ecol 38:226–228

Green TR, Ryan CA (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science 175:776–777

Jansen RMC, Wildt JJ, Hofstee JW, Bouwmeester HJ, van Henten EJ (2010) Plant volatiles: useful signals to monitor crop health status in greenhouses. In: Baluška F, Ninkovic V (eds) Plant communication from an ecological perspective. Springer, Berlin, pp 229–247

Karpinski S, Szechynska-Hebda M (2010) Secret life of plants: from memory to intelligence. Plant Signal Behav 5:1391–1394

Karpinski S, Reynolds H, Karpinska B, Wingsle G, Creissen G, Mullineaux P (1999) Systemic signaling and acclimation in response to excess excitation energy in Arabidopsis. Science 284:654–657

Khan ZR, Midega CA, Bruce TJ, Hooper AM, Pickett JA (2010) Exploiting phytochemicals for developing a 'push-pull' crop protection strategy for cereal farmers in Africa. J Exp Bot 61:4185–4196

Kobayashi Y, Yamamura N, Sabelis MW (2006) Evolution of talking plants in a tritrophic context: conditions for uninfested plants to attract predators prior to herbivore attack. J Theor Biol 243:361–374

Kumar AS, Lakshmanan V, Caplan JL, Powell D, Czymmek KJ, Levia DF, Bais HP (2012) Rhizobacteria *Bacillus subtilis* restricts foliar pathogen entry through stomata. Plant J 72:694–706

Lee B, Farag MA, Park HB, Kloepper JW, Lee SH, Ryu CM (2012) Induced resistance by a long-chain bacterial volatile: elicitation of plant systemic defense by a C13 volatile produced by *Paenibacillus polymyxa*. PLoS One 7:e48744

Lucas WJ, Yoo BC, Kragler F (2001) RNA as a long-distance information macromolecule in plants. Nat Rev Mol Cell Biol 2:849–857

Malone M (1992) Kinetics of wound-induced hydraulic signals and variation potentials in wheat seedlings. Planta 187:505–510

Masi E, Ciszak M, Stefano G, Renna L, Azzarello E, Pandolfi C, Mugnai S, Baluška F, Arecchi FT, Mancuso S (2009) Spatiotemporal dynamics of the electrical network activity in the root apex. Proc Natl Acad Sci USA 106:4048–4053

Molnar A, Melnyk C, Baulcombe DC (2011) Silencing signals in plants: a long journey for small RNAs. Genome Biol 12:215

Nagarajan VK, Jain A, Poling MD, Lewis AJ, Raghothama KG, Smith AP (2011) Arabidopsis Pht1;5 mobilizes phosphate between source and sink organs and influences the interaction between phosphate homeostasis and ethylene signaling. Plant Physiol 156:1149–1163

Palauqui JC, Elmayan T, Pollien JM, Vaucheret H (1997) Systemic acquired silencing: transgene-specific post-transcriptional silencing is transmitted by grafting from silenced stocks to non-silenced scions. EMBO J 16:4738–4745

Rodriguez-Saona CR, Mescher MC, De Moraes CM (2013) The role of volatiles in plant-plant interactions. In: Baluška F (ed) Long-distance systemic signaling and communication. Springer, Berlin

Ross AF (1966) Systemic effects of local lesion formation. In: Beemster ABR, Dijkstra J (eds) Viruses of plants. North-Holland, Amsterdam, pp 127–150

Rudrappa T, Biedrzycki ML, Kunjeti SG, Donofrio NM, Czymmek KJ, Paré PW, Bais HP (2010) The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. Commun Integr Biol 3:130–138

Ryan CA, Pearce G (2003) Systemins – a functionally defined family of peptide signals that regulate defensive genes in Solanaceae species. Proc Natl Acad Sci 100:14573–14577

Sampath Kumar A, Bais HP (2012) Wired to the roots: impact of root-beneficial microbe interactions on aboveground plant physiology and protection. Plant Signal Behav 7:1792–1798

Schwab R, Maizel A, Ruiz-Ferrer V, Garcia D, Bayer M, Crespi M, Voinnet O, Martienssen RA (2009) Endogenous TasiRNAs mediate non-cell autonomous effects on gene regulation in *Arabidopsis thaliana*. PLoS ONE 4:e5980

Sibaoka T (1999) Rapid plant movements triggered by action potentials. Bot Mag (Tokyo) 104:73–95

Smith AP, Nagarajan VK, Raghothama KG (2011) Arabidopsis Pht1;5 plays an integral role in phosphate homeostasis. Plant Signal Behav 6:1676–1678

Stahlberg R (2006) Historical overview of plant neurophysiology. Plant Signal Behav 1:6–8

Stahlberg R, Cleland RE, van Volkenburgh E (2005) Decrement and amplification of slow wave potentials during their propagation in *Helianthus annuus* L. shoots. Planta 220:550–558

Sticher L, Mauch-Mani B, Métraux JP (1997) Systemic acquired resistance. Annu Rev Phytopathol 35:235–270

Sun J-Q, Jiang H-L, Li C-Y (2011) Systemin/jasmonate-mediated systemic defense signaling in tomato. Mol Plant 4:607–615

Szechynska-Hebda M, Kruk J, Gorecka M, Karpinska B, Karpinski S (2010) Evidence for light wavelength-specific systemic photoelectrophysiological signalling and cellular light memory of excess light episode in Arabidopsis. Plant Cell 22:2201–2218

Ton J, D'Alessandro M, Jourdie V, Jakab G, Karlen D, Held M, Mauch-Mani B, Turlings TC (2007) Priming by airborne signals boosts direct and indirect resistance in maize. Plant J 49:16–26

Van Bel AJE, Hafke JB (2013) Calcium along the phloem pathway as a universal trigger and regulator of systemic alarms and signals. In: Baluška F (ed) Long-distance systemic signaling and communication. Springer, Berlin

van Wees SCM, de Swart, EAM, van Pelt JA, van Loon LC, Pieterse CMJ (2000) Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 97:8711–8716

Venkateshwaran M, Volkening JD, Sussman MR, Ané JM (2013) Symbiosis and the social network of higher plants. Curr Opin Plant Biol 16(1):118–127

Verheggen FJ, Haubruge E, Mescher MC (2010) Alarm pheromones-chemical signaling in response to danger. Vitam Horm 83:215–239

Wu Y, Zhang D, Chu JY, Boyle P, Wang Y, Brindle ID, De Luca V, Després C (2012) The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell Rep 1:639–647

Zimmermann MR, Maischak H, Mithöfer A, Boland W, Felle HH (2009) System potentials, a novel electrical long-distance apoplastic signal in plants, induced by wounding. Plant Physiol 149:1593–1600

Contents

Long-Distance Signaling in Systemic Acquired Resistance

Jyoti Shah and Ratnesh Chaturvedi

Abstract Systemic acquired resistance (SAR) is an inducible defense mechanism in plants that is activated throughout the foliage in response to a prior localized exposure to a foliar pathogen. The enhanced resistance status resulting from the activation of SAR can be maintained over a couple of generations. Critical to SAR is effective long-distance communication by the pathogen-inoculated organ with rest of the foliage, which requires the lipid transfer protein DIR1. The emerging consensus is that long-distance signaling in SAR involves networking between multiple vascular-translocated signaling molecules. The proposed salicylic acid receptor NPR1 is important for downstream signaling that involves defense priming. Chromatin remodeling is projected as an important mechanism in priming and memory associated with SAR.

Keywords Azelaic acid • Dehydroabietinal • Glycerol-3-phosphate • Methyl salicylate • Pipecolic acid • DIR1

1 Introduction

Plants utilize a combination of preformed and inducible defenses to control diseases (Spoel and Dong 2012). These defenses are manifested in the pathogen-infected organ and can also be activated systemically in tissues located distant to the site of initial infection. Systemic induction of disease resistance was reported as early as the 1930s (Chester 1933). Ross (1966) introduced the term systemic acquired resistance (SAR) to describe the enhanced state of resistance against viral infection in the upper leaves of tobacco (*Nicotiana tabacum*) plants that were previously inoculated on their lower leaves with *Tobacco mosaic virus* (TMV). SAR is now

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used more generally to refer to systemic enhanced resistance induced by prior local exposure to foliar pathogens (Sticher et al. 1997). SAR confers enhanced resistance against subsequent infection by a broad spectrum of pathogens, an effect that can extend to the roots (Gessler and Kuc 1982; Tahiri-Alaoui et al. 1993). SAR-conferred enhanced disease resistance is associated with the systemic induction of salicylic acid (SA) signaling and requires the *NPR1* (*NON-EXPRESSER OF PR GENES1*) gene (Chaturvedi and Shah 2007; Durrant and Dong 2004; Shah and Zeier 2013), which was recently demonstrated to be one of the receptors for SA (Wu et al. 2012). Light signaling mediated by the red/far-red light-absorbing phytochromes A and B is important for the SAR-associated systemic accumulation of SA and increase in disease resistance (Griebel and Zeier 2008; Zeier et al. 2004). The modulation of SAR strength by light is dependent on the *FMO1* (*FLAVIN-DEPENDENT MONOOXYGENASE1*) gene, which is also required for the SAR-associated systemic accumulation of SA (Mishina and Zeier 2006). In plants exhibiting SAR, defenses are primed to respond faster and stronger in response to challenge inoculation with pathogen (Conrath 2011). Recent studies with the model plant *Arabidopsis thaliana* indicate that once induced, the effect of SAR can be observed over a couple of generations (Luna et al. 2012). Systemic disease resistance in the foliar tissues is also observed in plants with roots colonized by beneficial rhizobacteria, a phenomenon termed induced systemic resistance (ISR) (van Loon 2007). However, SAR and ISR engage different defense mechanisms, and the combined activation of SAR and ISR has an additive effect on disease resistance in foliar tissues (van Wees et al. 2000). Similarly, mycorrhizal associations as well as biocontrol fungi also can promote disease resistance in the foliar tissues (Liu et al. 2007; Shoresh et al. 2010).

The activation of SAR requires long-distance signaling that facilitates communication with the systemic tissues by the organ experiencing the primary infection. The phloem is suggested to provide the conduit for translocation of the "systemic signal" involved in long-distance signaling. Girdling experiments in tobacco and grafting in cucumber (*Cucumis sativus*) suggested that the systemic signal is transported through the phloem (Guedes et al. 1980; Jenns and Kuc 1979; Tuzun and Kuc 1985). In Arabidopsis, the SAR-inducing activity is recovered in vascular sap-enriched petiole exudates (Pex) collected from pathogen-treated leaves (Chaturvedi et al. 2008; Jung et al. 2009; Maldonado et al. 2002). These Pexs are also effective in systemically enhancing disease resistance in other plant species (Chaturvedi et al. 2008). Experiments in *Arabidopsis* indicated that the SAR signal may not be exclusively transported through the phloem, since systemic expression of the *PR1* (*PATHOGENESIS-RELATED1*) gene, which is a molecular marker for SAR, was not limited to the tissues connected by the path of photoassimilate translocation from the primary-infected organ (Kiefer and Slusarenko 2003).

SA levels increase in the phloem sap during SAR (Malamy et al. 1990; Métraux et al. 1990). Hence, for a long time SA was thought to be the systemic signal in SAR (Uknes et al. 1992; Yalpani et al. 1991). However, grafting studies involving tobacco plants expressing the *Pseudomonas putida nahG* gene-encoded salicylate hydroxylase, an enzyme that converts salicylic acid to catechol, confirmed that although required for the manifestation of SAR-conferred enhanced disease

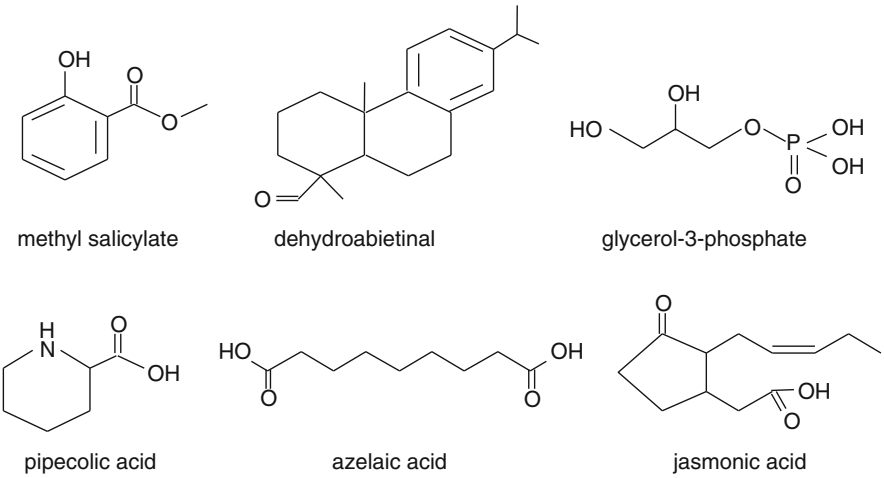

Fig. 1 Structures of metabolites putatively involved in long-distance signaling associated with systemic acquired resistance

resistance, SA per se was not the systemic signal in SAR (Vernooij et al. 1994). Similar conclusions were arrived at with experiments involving tobacco plants rendered SA deficient due to epigenetic suppression of phenylalanine ammonia-lyase expression (Pallas et al. 1996). In recent years, several novel metabolites (Fig. 1) involved in systemic signaling leading to SAR have been described, leading to the suggestion that multiple systemic signals are likely involved in SAR (Depmsey and Klessig 2012; Shah 2009; Shah and Zeier 2013). This chapter will summarize evidence supporting the involvement of these molecules in long-distance communication by the primary-infected organ and the ensuing signaling in the systemic tissues during SAR. Readers are directed to Table 1 for a list of genes that are involved in the synthesis and/or signaling mediated by these SAR signals and biologically (pathogen inoculation) induced SAR.

2 Plant Metabolites Involved in Long-Distance Signaling in SAR

2.1 Methyl Salicylate

The role of methyl salicylate (MeSA; Fig. 1) in long-distance signaling in SAR was first reported for tobacco. Increases in MeSA levels were observed in TMV-infected leaves of a TMV-resistant tobacco cultivar (Park et al. 2007). A parallel increase in MeSA was also observed in Pex collected from the TMV-infected leaves and in the systemic leaves. It was noted that the SA-binding protein

Table 1 Plant genes involved in SAR

Gene	Plant	AtG#	Function
NPR1	*A. thaliana*	At1g64280	SA receptor; transcription coactivator
NPR3	*A. thaliana*	At5g45110	SA receptor involved in proteasomal turnover of NPR1
NPR4	*A. thaliana*	At4g19660	SA receptor involved in proteasomal turnover of NPR1
FMO1	*A. thaliana*	At1g19250	Required for systemic SA accumulation
PHYA	*A. thaliana*	At1g09570	Red/far-red light perception; required for light's influence on SAR
PHYB	*A. thaliana*	At2g18790	Red/far-red light perception; required for light's influence on SAR
MES9	*A. thaliana*	At4g37150	MeSA esterase
BSMT1	*A. thaliana*	At3g11480	Benzoic acid/salicylic acid methyl transferase; synthesizes MeSA
ICS1 (*SID2*)	*A. thaliana*	At1g74710	Isochorismate synthase activity involved in SA synthesis
SFD1 (*GLY1*)	*A. thaliana*	At2g40690	Dihydroxyacetonephosphate reductase; synthesizes glycerol-3-phosphate in plastids
DIR1	*A. thaliana*	At5g48485	Lipid-transfer protein
AZI1	*A. thaliana*	At4g12470	Putative lipid-transfer protein
ALD1	*A. thaliana*	At2g13810	Aminotransferase required for pipecolic acid synthesis
ACP4	*A. thaliana*	At4g25050	Acyl-carrier protein required for cuticle development
ACBP3	*A. thaliana*	At4g24230	Acyl-CoA-binding protein required for cuticle development
ACBP4	*A. thaliana*	At3g05420	Acyl-CoA-binding protein required for cuticle development
ACBP6	*A. thaliana*	At1g31812	Acyl-CoA-binding protein required for cuticle development
MPK3	*A. thaliana*	At3g45640	MAP-kinase
MPK6	*A. thaliana*	At2g43790	MAP-kinase
HSFB1	*A. thaliana*	At4g36990	Putative DNA binding protein
NtSABP2	*N. tabaccum*	–	SA-binding protein with MeSA esterase activity
NtSAMT1	*N. tabaccum*	–	SA-methyl transferase; biological synthesis of MeSA
StMES1	*S. tuberosum*	–	Methyl Esterase; release SA from MeSA

SABP2, which is required in the systemic leaves for the activation of SAR, possessed MeSA esterase activity (Forouhar et al. 2005; Kumar et al. 2006; Park et al. 2007), thereby suggesting that MeSA hydrolysis in the systemic leaves may have a role in SAR (Park et al. 2007). Indeed, genetic studies confirmed that MeSA esterase activity of SABP2 was essential for its involvement in SAR; a $Ser_{81} \rightarrow Ala_{81}$ missense mutation that abolished SABP2's MeSA esterase activity was unable to complement the SAR defect of a transgenic line in which expression of the endogenous *SABP2* gene was silenced (Park et al. 2007). Pharmacological experiments provided additional support for the importance of SABP2's MeSA esterase activity in SAR. 2,2,2,2′-tetra-fluoroacetophenone, a competitive inhibitor of SABP2's esterase activity, when applied to wild-type plants blocked the

activation of SAR (Park et al. 2009). It was suggested that the conversion of MeSA to SA in the systemic leaves was critical for SAR (Park et al. 2007).

MeSA is synthesized by *S*-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase, which catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine to SA. RNAi-mediated silencing of the tobacco *SAMT1* (*SA-METHYLTRANSFERASE1*) gene attenuated MeSA accumulation in response to TMV infection and also compromised SAR (Park et al. 2007). Genetic studies indicated that unlike *SABP2*, *SAMT1* function in SAR was required in the primary pathogen-treated organs. In grafting experiments, it was observed that SAR was compromised in the wild-type scion that was grafted on a *NtSAMT1*-silenced root stock, which received the primary TMV inoculation. By contrast, the *NtSAMT1*-silenced scions grafted on a wild-type root stock were SAR competent. It was therefore concluded that MeSA synthesized by SAMT1 in the primary pathogen-inoculated leaves is transported via the phloem to the systemic leaves of tobacco, where it is processed by the esterase activity of SABP2 to yield SA, which is biologically active to trigger SAR (Depmsey and Klessig 2012).

MeSA levels also increased in the pathogen-inoculated and systemic leaves of potato (*Solanum tuberosum*) and *Arabidopsis* plants exhibiting SAR (Manosalva et al. 2010; Vlot et al. 2008). In potato, 2,2,2,2′-tetrafluoroacetophenone application blocked arachidonic acid-induced SAR (Manosalva et al. 2010). Furthermore, RNAi-mediated silencing of the potato *METHYL ESTERASE 1* (*StMES1*) gene, which encodes a SABP2 homolog, compromised arachidonic acid-induced SAR (Manosalva et al. 2010). Similarly, 2,2,2,2′-tetrafluoroacetophenone applied to the systemic leaves inhibited SAR in *Arabidopsis* (Park et al. 2009). SAR was also curtailed in *Arabidopsis* plants in which expression of multiple *AtMES* genes encoding putative MeSA esterases was depressed (Vlot et al. 2008). However, this effect was observed only in 50 % of experiments (Chaturvedi et al. 2012; Vlot et al. 2008), suggesting that the involvement of MeSA in SAR is influenced by other factors. Similarly, while one study demonstrated that *Arabidopsis bsmt1* (*benzoic acid/salicylic acid methyl transferase 1*) mutants, which are deficient in MeSA synthesis, were SAR competent (Attaran et al. 2009), studies by another group demonstrated that SAR was weaker in *bsmt1* mutant plants (Liu et al. 2010, 2011a), providing additional support to the conditional requirement of MeSA in SAR. Liu et al. (2011a) have suggested that light is a likely factor contributing to this variable need of MeSA in SAR. They demonstrated that the time of the day when the plant is inoculated with a SAR-inducing pathogen determines the relative importance of MeSA in SAR. When the inoculations were done closer to the start of the dark period, MeSA was required for SAR. However, when the inoculations were done earlier during the light period, MeSA was less important.

2.2 Dehydroabietinal

Chaturvedi et al. (2012) used a biochemical approach to purify the systemic resistance-inducing activity from *Arabidopsis* AvrPex. Their efforts resulted in

the identification of dehydorabietinal (DA) (Fig. 1), an abietane diterpenoid, as a potent inducer of SAR (Chaturvedi et al. 2012). Terpenoids include a large group of plant metabolites that have varied functions in plant growth and development, and interaction with other organisms. Picomolar solutions of chemically synthesized DA when applied to a few leaves of *Arabidopsis* induced systemic disease resistance against the bacterial pathogen *Pseudomonas syringae* and the fungal pathogen *Fusarium graminearum* (Chaturvedi et al. 2012). At these low concentrations, DA did not function as an antibiotic, thus suggesting its effect on limiting pathogen growth in plants was indirect.

Locally applied deuterium-labeled DA was systemically transported through *Arabidopsis*. DA application resulted in the local and systemic induction of SA accumulation and expression of the SA-responsive *PR1* gene (Chaturvedi et al. 2012). *FMO1*, which is required for the systemic accumulation of SA during biologically induced SAR (Mishina and Zeier 2006), was also required for the DA-induced systemic increase in SA. However, *FMO1* was not required for SA accumulation in the DA-treated leaves. DA was unable to induce systemic disease resistance in the *fmo1* mutant and in the isochorismate synthase-deficient *ics1 ics2* double mutant, which lacks the ability to synthesize SA via the isochorismate pathway, thus suggesting that DA-induced systemic disease resistance requires SA accumulation. In agreement with the requirement of SA for DA-induced systemic disease resistance, DA was unable to promote resistance in transgenic plants expressing the *nahG* gene. The proposed SA receptor NPR1 (Wu et al. 2012) was required for DA-induced systemic resistance, thus confirming that DA-conferred systemic disease resistance is due to the activation of SAR. Biologically induced SAR was not accompanied by an increase in DA content. Rather, during the biological induction of SAR, DA was redistributed from a biologically inactive (unable to induce SAR) form that elutes in a low-molecular weight range (<30 kDa) to a signaling form (DA*) that is SAR competent and elutes at a higher molecular weight range (>100 kDa). Trypsin treatment abolished the SAR-inducing capabilities of DA* (Chaturvedi et al. 2012), thus suggesting that DA* in AvrPex is associated with one or more proteins that are required for DA-induced systemic disease resistance.

Besides *Arabidopsis*, DA is also present in tobacco and tomato (*Solanum lycopersicum*), and DA application promoted systemic disease resistance in these species, suggesting that DA's role in defense is likely conserved in plants (Chaturvedi et al. 2012). However, whether biological induction of SAR requires DA is currently not known and requires experimentation with plants in which DA accumulation, in particular DA*, is blocked. Although the biosynthesis pathway for DA in angiosperms remains to be elucidated, clues on the synthesis of DA can be drawn from the biosynthesis of abietane family of diterpenoids in conifers, where these metabolites are synthesized by a mechanism that is similar to the biosynthesis of gibberellins (Bohlmann and Keeling 2008; Tholl 2006; Trapp and Croteau 2001).

2.3 A Glycerol-3-Phosphate-Derived Factor

Genetic studies in *Arabidopsis* have implicated the involvement of a glycerol-3-phosphate (G3P)-dependent factor in long-distance SAR signaling. Biologically-induced SAR was compromised in mutant plants lacking *SFD1* (*SUPPRESSOR OF FATTY ACID DESATURASE DEFICIENCY1*) activity (Nandi et al. 2004). The systemic increase in SA and *PR1* expression that accompanies SAR was also attenuated in the *sfd1* mutant compared to the wild-type plant (Nandi et al. 2004). AvrPex collected from the *sfd1* mutant were unable to induce systemic disease resistance, when applied to wild-type plants, suggesting that SFD1 activity is required for the synthesis and/or translocation of a long-distance SAR signal (Chaturvedi et al. 2008). By comparison, SAR was restored in *sfd1* mutant plants treated with AvrPex from wild-type plants, thus indicating that the *sfd1* mutant is responsive to the long-distance SAR signal. SFD1 is a dihyroxyacetone phosphate (DHAP) reductase that catalyzes the synthesis of G3P (Fig. 1) from DHAP. G3P is an important precursor for a variety of biomolecules, including membrane and storage lipids. The *sfd1* mutant contained lower level of 34:6-monogalactosyldiacylglycerol, a major galactolipid in *Arabidopsis* that is synthesized in the plastids. This decrease in 34:6-monogalactosyldiacylglycerol was accompanied by a compensatory increase in 36:6-monogalactosyldiacylglycerol. Missense mutations that abolish SFD1's DHAP reductase activity were unable to complement the 34:6-monogalactosyldiacylglycerol deficiency and the SAR defect of the *sfd1* mutant, indicating that SFD1's involvement in galactolipid synthesis and SAR requires its DHAP reductase activity (Lorenc-Kukula et al. 2012). SFD1 contains a leader sequence at its N-terminus that is required for targeting SFD1 to the plastids. Although SFD1 lacking this leader sequence retains DHAP reductase activity, the N-terminus-deleted SFD1 was unable to complement the SAR and 34:6-monogalactosyldiacylglycerol deficiency of *sfd1*, suggesting that SFD1's DHAP reductase activity is required in the plastids for SAR and galactolipid synthesis (Lorenc-Kukula et al. 2012).

More recently SAR was also shown to be compromised in the *gly1* mutant, which is allelic with *sfd1* (Chanda et al. 2011). However, unlike the *sfd1* mutants, which are in the accession Nössen, systemic increase in SA and *PR1* expression that accompanies SAR was not attenuated in the *gly1* mutant, which is in the accession Columbia. Chanda et al. (2011) demonstrated that SAR was accompanied by an increase in G3P content in leaves treated with a SAR-inducing pathogen. G3P levels were also elevated in AvrPex and in the distal leaves of these plants. Local application of G3P with AvrPex or Avr pathogen restored SAR in the *gly1* mutant (Chanda et al. 2011). These pharmacological studies along with the genetic studies with plants expressing DHAP reductase-deficient SFD1 (Lorenc-Kukula et al. 2012), confirm an important role for SFD1-derived G3P in SAR. Chanda et al. (2011) further noted that ^{14}C-labeled G3P infiltrated into *Arabidopsis* leaves could not be recovered in the distal leaves as [^{14}C]G3P. Thus, G3P per se is likely not systemically translocated and the systemic increase in G3P observed during SAR is likely due to the de novo synthesis of G3P in the distal leaves. Further work is

needed to identity the G3P-dependent factor associated with long-distance signaling in SAR.

G3P when applied by itself to wild-type *Arabidopsis* was not sufficient to induce systemic resistance. However, when co-applied with Pex from either $MgCl_2$-treated or Avr pathogen-inoculated plants, G3P was capable of enhancing systemic disease resistance, thus suggesting that a factor present in Pex is required for G3P-promoted systemic disease resistance (Chanda et al. 2011). Local application of G3P resulted in the enhanced expression of the *MES9* gene in the systemic tissues of *Arabidopsis* (Chanda et al. 2011). *MES9* encodes a homolog of the tobacco SABP2. By comparison, expression of the *BSMT1* gene was downregulated, thus predicting increased conversion of MeSA to SA in the systemic leaves of plants that were locally treated with G3P. However, comparable to plants that received a local control (mock) treatment, no increase in SA or SAG was observed in the systemic leaves of *Arabidopsis* that were treated on other leaves with G3P (Chanda et al. 2011). Thus the significance of the altered expression of *MES9* and *BSMT1* to G3P-induced SAR is unclear.

2.4 Azelaic Acid

As mentioned above, in tissues exhibiting SAR, defenses are primed to respond faster and stronger in response to pathogen infection. However, how these defenses are primed is poorly understood. Jung and coworkers (2009) suggested that azelaic acid (Fig. 1), a nine-carbon dicarboxylic acid, is involved in priming of systemic defenses. GC-MS scans for small molecules (70–550 Da) revealed elevated levels of azelaic acid in AvrPex compared to Pex collected from mock-treated *Arabidopsis* leaves (Jung et al. 2009). Locally applied deuterium-labeled azelaic acid could be recovered in PeX and the distal leaves, indicating that azelaic acid is systemically transported. When applied at concentrations greater than 10 μM, azelaic acid systemically enhanced disease resistance. Azelaic acid-induced systemic resistance in *Arabidopsis* required genes involved in SA synthesis and signaling, *DIR1* (*DEFECTIVE IN INDUCED RESISTANCE1*), *FMO1*, and *ALD1* (*AGD2-LIKE DEFENSE RESPONSE PROTEIN1*), which encodes an aminotransferase that is involved in pipecolic acid synthesis (see Sect. 2.5). However, unlike MeSA and DA, azelaic acid applied to *Arabidopsis* foliage did not increase SA content and *PR1* expression. Instead, azelaic acid-treated plants were primed for the enhanced accumulation of SA and *PR1* expression when challenged with a pathogen. Although azelaic acid treatment did not have a major impact on the plant transcriptome, one of the genes that was transiently expressed at elevated levels in azelaic acid-treated plants was *AZI1* (*AZELAIC ACID-INDUCED 1*) (Jung et al. 2009), which encodes a protein with homology to lipid-transfer proteins. *AZI1* expression was also induced in leaves treated with AvrPex (Jung et al. 2009). *AZI1* is required for priming associated with azelaic acid- and biologically induced SAR.

A likely mechanism for the synthesis of azelaic acid involves the sequential action of 9-lipoxygenase (9-LOX) and hydroperoxide lyases on fatty acids to yield 9-oxononanoic acid that is subsequently oxidized to yield azelaic acid. To determine if *LOX1* and *LOX5*, the two 9-LOX-encoding genes in *Arabidopsis*, are involved in pathogen infection associated accumulation of azelaic acid, Zoeller et al. (2012) compared azelaic acid levels in the *Arabidopsis lox1 lox5* double mutant plant after inoculation with pathogen. However, azelaic acid levels were found to increase to comparable levels in the pathogen-inoculated leaves of wild-type and the *lox1 lox5* plant, suggesting that *LOX1* and *LOX5* do not contribute to azelaic acid synthesis in pathogen-inoculated *Arabidopsis*. Instead, it was suggested that azelaic acid is synthesized in plastids by a free radical-based galactolipid fragmentation mechanism (Zoeller et al. 2012). Zoeller and coworkers (2012) further suggested that azelaic acid is a general marker for lipid peroxidation.

2.5 Pipecolic Acid

In addition to azelaic acid, the lysine catabolite pipecolic acid (Pip) (Fig. 1) also has been implicated in priming and amplification of plant defenses that contribute to SAR-conferred enhanced disease resistance. In addition, Pip is also required for local defenses against virulent and avirulent pathogen (Návarová et al. 2012). The levels of Pip increase in the pathogen-inoculated and the systemic pathogen-free leaves. Pip application promotes local and systemic disease resistance in *Arabidopsis*. Pip accumulation in *Arabidopsis* infected with pathogen requires the *ALD1*-encoded aminotransferase, which is also required for SAR (Jing et al. 2011; Song et al. 2004a, b). Pip application restored disease resistance in the *ald1* mutant. *ALD1* expression is induced in pathogen-infected and systemic leaves. Since lysine can be utilized as a substrate by ALD1 in vitro (Návarová et al. 2012), the aminotransferase activity of ALD1 likely is directly involved in Pip synthesis in vivo.

Pip also accumulates at elevated levels in Pex collected from pathogen-inoculated leaves; thus Pip could be systemically transported. The low level of Pip that accumulates in the systemic uninfected leaves of plants exhibiting SAR likely promotes its own synthesis when challenged with pathogen by inducing *ALD1* expression. Since *ALD1* is also involved in a SA amplification loop (Song et al. 2004b), Pip therefore might contribute to signal amplification by priming SA accumulation in response to challenge with pathogen. Indeed, pre-treatment with Pip resulted in a faster increase in SA content in response to subsequent pathogen inoculation. *FMO1*, which is required for systemic accumulation of SA, is also required for the systemic induction of *ALD1* expression during SAR and for Pip-induced systemic disease resistance, leading to a model in which Pip acting through *FMO1*, promotes *ALD1* expression and thus its own synthesis in the distal leaves, thereby priming the rapid increase in SA content upon pathogen infection.

2.6 *Jasmonates*

Jasmonic acid (JA) (Fig. 1) and its derivatives, collectively known as jasmonates, are involved in systemic signaling associated with wounding in tomato (Lee and Howe 2003) and have also been suggested to be involved in the manifestation of SAR (Truman et al. 2007). The *OPR3* (*12-OXOPHYTODIENOATE REDUCTASE 3*) gene, which is involved in JA synthesis, and the *JIN1* and *JAI4* genes, both of which are associated with JA signaling, were required for the activation of SAR in *Arabidopsis* (Truman et al. 2007). Furthermore, JA rapidly accumulated in the AvrPex. This accumulation of JA in AvrPex was paralleled by the systemic induction of JA-responsive genes and preceded the expression of the SA-responsive genes. MeJA application induces expression of the *Arabidopsis BSMT1* gene (Koo et al. 2007), thus suggesting that jasmonates could potentially promote MeSA synthesis in the primary pathogen-inoculated leaves and thus contribute to long-distance signaling in SAR. However, results from other studies have questioned the involvement of JA as a systemic signal in SAR. Unlike Truman et al. (2007), Attaran et al. (2009) reported that the *opr3* and *jin1* mutants were SAR competent. The ability to induce SAR was also retained in the JA-insensitive *coi1* (*coronatine insensitive1*) and the *jar1* (*jasmonate resistant1*) mutants (Attaran et al. 2009; Cui et al. 2005; Mishina and Zeier 2007). Furthermore, when AvrPex was fractionated by molecular-sieve chromatography, JA did not copurify in fractions that contained the SAR-inducing activity (Chaturvedi et al. 2008). It is plausible that other environmental factors might influence the involvement of JA in SAR, thus explaining the differences between Truman et al. (2007) and the other studies.

3 Lipid-Transfer Proteins in Long-Distance Signaling

3.1 *DIR1*

The *Arabidopsis DIR1* was one of the first genes to be identified that is critical for long-distance signaling in SAR (Maldonado et al. 2002). The *Arabidopsis dir1* mutant was incapable of developing SAR in response to primary inoculation with an Avr pathogen. AvrPex collected from the *dir1* mutant were unable to systemically enhance *PR1* expression and disease resistance when applied to wild-type plants (Chaturvedi et al. 2008; Maldonado et al. 2002). However, the *dir1* mutant was responsive to the SAR signal present in AvrPex collected from wild-type plants, thus suggesting that *DIR1* is required for the accumulation and/or long-distance translocation of a SAR signal(s) (Chaturvedi et al. 2008; Maldonado et al. 2002). Basal resistance against pathogen was not impacted in the *dir1* mutant (Maldonado et al. 2002), thus suggesting that DIR1 is specifically required for SAR. More recently, DIR1 homologs were also demonstrated to have an important function in SAR in tobacco, as well (Liu et al. 2011b).

Liu et al. (2011b) demonstrated that the *Arabidopsis BSMT1* gene, which encodes a MeSA-synthesizing methyltransferase, was expressed at elevated levels in the Avr pathogen-inoculated and the systemic leaves of the *dir1* mutant compared to the wild-type plant. Furthermore, MeSA content was higher and free SA and its glucoside, SAG, levels were lower in these *dir1* tissues, thus suggesting that in the systemic leaves of wild-type plants, DIR1 depresses the conversion of SA to MeSA, and thereby promotes SA accumulation. Similarly, in tobacco silenced for expression of the *DIR1* gene, a correlation was observed between the SAR-deficient phenotype and elevated *SAMT1* expression level in the pathogen-treated and distal tissues (Liu et al. 2011b).

Overexpression of DIR1 in *Arabidopsis* did not lead to the constitutive activation of SAR-like responses (Maldonado et al. 2002). This suggests that additional factors are required for systemic signaling in SAR. Unlike AvrPex from the *dir1* and *sfd1* mutants, which when applied individually to wild-type plants were unable to induce SAR, when mixed together, *dir1* plus *sfd1* AvrPexs were effective in systemically inducing *PR1* expression and disease resistance (Chaturvedi et al. 2008). These results implicate a combined requirement of DIR1 and a G3P-dependent factor in SAR. In support of this view, Chanda et al. (2011) observed that DIR1 when co-applied with G3P was capable of inducing SAR in *Arabidopsis*. It was suggested that G3P promotes the systemic translocation of DIR1 (Chanda et al. 2011). In light of the observations that G3P promotes the expression of *MES*9 and depresses the expression of *BSMT1* in *Arabidopsis* (Chanda et al. 2011), and *DIR1* promotes systemic SA accumulation (Liu et al. 2011b), it would be important to know whether co-application of DIR1 + G3P impacts systemic accumulation of SA and *PR1* expression. DIR1 is also required for DA- and azelaic acid-induced SAR (Chaturvedi et al. 2012; Jung et al. 2009). Likewise, *SFD1*, and hence presumably a G3P-dependent factor, is also required for the full potential of DA-induced SAR.

The crystal structure of DIR1 indicates that it shares some similarities to LTP2 family of lipid-transfer proteins (Lascombe et al. 2008). Recombinant DIR1 can bind lipids. DIR1 contains two SH3 domains, which in other proteins facilitate protein–protein interaction. Lascombe et al. (2008) suggested that DIR1 likely interacts with other proteins, as well. Indeed, compared to the relatively small size of DIR1 (<10 kDa), the SAR-inducing activity in AvrPex, which is trypsin sensitive, elutes in a range that is larger than 100 kDa (Chaturvedi et al. 2012). DIR1 is present in this SAR activating fraction derived from AvrPex (R. Chaturvedi and J. Shah, unpublished), thus supporting the opinion that DIR1 associates with other proteins.

3.2 *AZI1*

As mentioned above, expression of *AZI1*, which encodes a putative lipid-transfer protein, is induced in AvrPex- and azelaic acid-treated leaves (Jung et al. 2009).

Biologically activated, and AvrPex- and azelaic acid-induced systemic disease resistance was compromised in the *azi1* mutant (Chaturvedi et al. 2012; Jung et al. 2009). The SAR-associated priming of SA accumulation and *PR1* expression were weaker in the *azi1* mutants than the wild-type plant (Jung et al. 2009). Azelaic acid and AvrPex when applied to the *azi1* mutant were capable of inducing disease resistance in the treated leaves, thus indicating that the *azi1* mutant is sensitive to azelaic acid and the SAR-inducing signal present in AvrPex. By comparison, AvrPex collected from the *azi1* mutant were unable to enhance disease resistance in the foliage of wild-type plants, thus suggesting that *AZI1* is likely involved in the production and/or the translocation of a long-distance signal involved in defense priming. Whether the local accumulation and/or systemic translocation of azelaic acid or any of the other signaling metabolites described above is impacted in the *azi1* mutant, remains to be determined. Although not essential for systemic disease resistance induced by DA applied at concentrations above 10 pM, *AZI1* was required for systemic disease resistance induced by lower concentrations of DA, thus suggesting that *AZI1*- and azelaic acid-mediated priming promotes DA's effectiveness in inducing SAR. It would be of particular interest to determine if AZI1 is part of the high-molecular weight complex that contains DA* and DIR1.

4 Perception of the SAR Signals and Ensuing Signaling

4.1 Perception of the SAR Signals

How some of the systemic signals are perceived in the systemic leaves is not known. In case of MeSA, binding to MeSA esterase might be a mechanism by which MeSA is perceived during SAR. As discussed below, an intact cuticle has been suggested to be important for perception of the SAR signal.

The cuticle, which is composed of waxes and cutin monomers, forms a hydrophobic barrier on the surface of most foliar tissues. The cuticle also provides a physical barrier to pathogens. It has also been suggested to serve as a source for signals that promote resistance against the necrotrophic fungus *Botrytis cinerea* (Chassot and Métraux 2005; Chassot et al. 2007). In other cases, damaged cuticle has been associated with increased susceptibility to pathogens (Xia et al. 2012). An intact cuticle has also been demonstrated to be required for SAR. SAR was compromised when the cuticle was mechanically damaged in *Arabidopsis* (Xia et al. 2009). Furthermore, SAR was compromised in the cuticle-defective *acp4* (*acyl carrier protein 4*) mutant. The *acp4* mutant was impaired in its ability to respond to the SAR signal present in AvrPex from wild-type plants (Xia et al. 2009, 2010). In comparison, AvrPex from *acp4* was capable of inducing SAR when applied to wild-type plants, thus indicating that the *acp4* mutant is capable of producing the systemically translocated SAR signal, but is deficient in the perception and/or response to this signal. Mutations in some genes encoding acyl

CoA-binding proteins (ACBPs) that are required for proper cuticle development also resulted in attenuated SAR (Xia et al. 2012). However, unlike the *acp4* mutant, these *acbp* mutants were responsive to the SAR signal. Instead, AvrPex from these *acbp* mutants lacked the ability to induce SAR when applied to wild-type leaves. Xia et al. (2012) have suggested that these *acbp* mutants are defective in the generation of the long-distance SAR signal. Thus, cuticular components could be involved in both, signal generation and perception.

AZI1 expression is also elevated in transgenic *Arabidopsis* with cuticular defects resulting from expression of a fungal cutinase (Chassot et al. 2007). Whether *AZI1* expression is similarly altered in the *acp4* and/or the *acpb* mutants remains to be determined. However, the *acbp* mutants were not affected in pathogen-induced accumulation of azelaic acid (Xia et al. 2012), thus indicating that their SAR-deficiency is not due to defects in azelaic acid accumulation. It remains to be determined if the different classes of cuticular mutants have defects in the accumulation and/or response to one or more of the long-distance signaling molecules reviewed here.

4.2 Downstream Signaling in SAR

4.2.1 Priming

Priming involves mechanisms that make the primed cells more sensitive to perceive and/or respond to a stress, than non-primed cells (Conrath 2011). A primed state is also one of the characteristics of SAR. During SAR, SA accumulation and SA signaling are primed to respond more strongly when the tissue is challenged by a pathogen. Azelaic acid and Pip have been implicated in priming increases in SA content in response to challenge inoculation with pathogen (Jung et al. 2009; Návarová et al. 2012). The *FMO1* gene, which is required for azelaic acid- and Pip-induced SAR, has been suggested to participate in a feedback loop involving Pip and the *ALD1* gene to promote SA accumulation during SAR (Návarová et al. 2012).

Recent studies indicate that priming in SAR is associated with alterations in MAPK pathway activity and also epigenetic alterations of transcription regulatory genes. *MPK3* and *MPK6* transcripts and the corresponding proteins accumulated at elevated levels in the systemic tissues of *Arabidopsis* in which SAR was induced by inoculating the lower leaves with an Avr pathogen (Beckers et al. 2009). When infiltrated with water or pathogen, the levels of MPK3 and MPK6 proteins increased further in the systemic leaves of plants exhibiting SAR, than control plants in which SAR was not induced. This increase in MPK3 and MPK4 correlated with the higher expression of *PR1* and systemic disease resistance. The primed expression of *PR1* was not observed in the *mpk3* mutants, thus confirming the involvement of *MPK3* in priming associated with SAR. By contrast to MPK3, MPK6 had a weaker contribution to priming in SAR. The SA receptor NPR1 was

required for this priming. MPK3 and MPK4 transcript and protein also accumulate at elevated levels when low levels of the SA analogue benzo(1,2,3)thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) were applied to *Arabidopsis*. The requirement of NPR1 and the ability of BTH to prime MPK3 and MPK6 are suggestive of the involvement of SA signaling in this priming of MPKs. Whether azelaic acid and Pip involvement in priming involves MPK3 and MPK6 is not known.

Heat shock factors (HSFs) are DNA binding proteins that are involved in regulating expression of the heat shock proteins. In *Arabidopsis*, the *HSFB1* gene (also referred as *TBF1* and *HSF4*) is required for SA-induced disease resistance (Pajerowska-Mukhtar et al. 2012) and for priming defense gene expression during SAR (Pick et al. 2012). Expression of *HSFB1* was upregulated in the systemic leaves of plants that were inoculated on their lower leaves with Avr strains of *P. syringae*. *hsfb1* mutant plants were defective in the BTH-induced priming of the defense genes *PAL1* (*PHENYLALANINE AMMONIA-LYASE1*) and *WRKY29*. Furthermore, local inoculation with an Avr pathogen was unable to enhance systemic disease resistance in the *hsfb1* mutant plants. Since *PR1* expression was induced in the systemic leaves, but disease resistance was not, and BTH was unable to prime gene expression in the *hsfb1*plants, Pick et al. (2012) suggested that the *hsfb1* mutant is not defective in systemic long-distance signaling, but rather in the priming of defenses associated with SAR.

Chromatin remodeling involving histone modifications has also been implicated in priming and memory associated with SAR. Jaskiewicz et al. (2011) observed that *Arabidopsis* plants that were inoculated on their lower leaves with a pathogen exhibited enhanced level of histone modifications on the *WRKY6*, *WRKY29*, and *WRKY53* genes. Despite these chromatin modifications, expression of these three genes was not substantially altered in the systemic pathogen-free leaves. However, when these systemic leaves were stressed by infiltrating water, expression of these *WRKY* genes increased substantially over that in the non-primed plants. Similarly, BTH-promoted priming and stress-induced expression of these genes were accompanied by increased histone modifications. The *NPR1* gene was required for the increase in histone modifications and for the stress-induced expression of these genes in response to pre-treatment with BTH, thus confirming a role for *NPR1* in BTH-promoted chromatin modification and in priming the stress-induced expression of these *WRKY* genes. Readers are directed to two excellent reviews (Conrath 2011; van den Burg and Takken 2009) on the involvement of chromatin modification in basal and induced expression of SA-responsive genes and in defense priming. Determining whether there is a relationship between histone modifications and the MAPK cascade, and azelaic acid- and Pip-promoted priming in SAR will be of particular interest.

4.2.2 The SA Receptors

The NPR1 protein exists in the nucleus and the cytosol. In the cytosol, NPR1 is suggested to exist as an oligomer. Its conversion to the monomeric form promotes

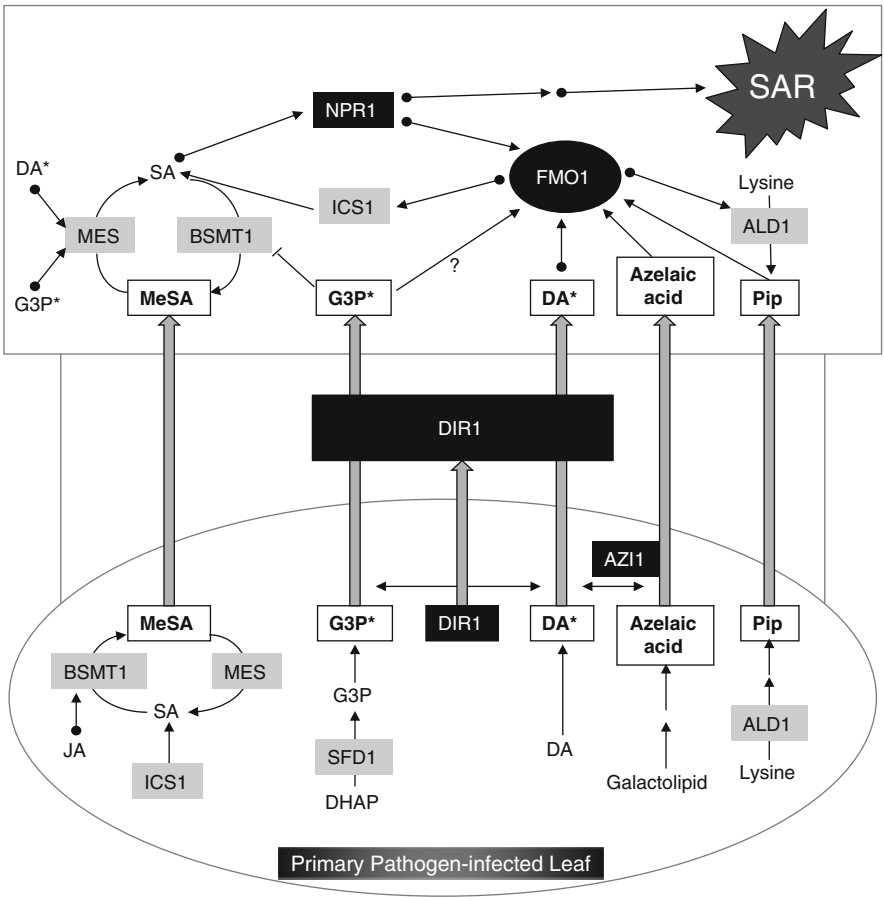

Fig. 2 Networking between SAR signaling molecules. *Events in the primary pathogen-infected leaf:* Increased activity of ICS1, resulting from increased expression of the corresponding gene, contributes to increase in SA content. SA is converted to MeSA by BSMT1. In tobacco, the high level of SA was simultaneously shown to inhibit the MeSA esterase (MES) activity of SABP2, thus ensuring increase in MeSA level. JA is known to promote expression of *BSMT1*. Simultaneously, glycerol-3-phosphate levels increase. SFD1 (GLY1) catalyzes the synthesis of glycerol-3-phosphate from dihydroxyacetone phosphate (DHAP). Azelaic acid and pipecolic (Pip) levels also increase. Azelaic acid has been suggested to be synthesized on galactolipids by a nonenzymatic method, while Pip synthesis from lysine requires the ALD1 aminotransferase. Expression of the *ALD1* gene is induced in response to pathogen inoculation. Absolute levels of DA do not change. However, DA is mobilized from a non-signaling low-molecular weight to a high-molecular weight signaling DA (DA*) complex in response to pathogen inoculation. Trypsin treatment destroys the high-molecular weight DA* complex, suggesting the presence of proteins in this complex. DIR1 is one of the proteins in this high-molecular weight complex. The azelaic acid-inducible *AZI1* gene is required for accumulation and/or transport of the SAR signal. *AZI1* is required for azelaic acid-induced SAR and also promotes DA*-induced SAR. However, its involvement in SAR induced by the other factors is not known. *Events in the distal (systemic) leaf:* Systemic transport of MeSA, a G3P-derived factor (G3P*), DA*, azelaic acid, Pip, and DIR1 from the pathogen-inoculated leaf to the distal leaves occurs via the vasculature, most probably the

NPR1s translocation into the nucleus (Mou et al. 2003). It is the nuclear form of NPR1 that is critical for SA-induced expression of *PR1*, by functioning as a transcription coactivator. Wu et al. (2012) recently suggested that NPR1 is one of the receptors of SA. Using equilibrium dialysis approach, they demonstrated that NPR1 could bind [^{14}C]SA with an apparent K_d of 140 nM. They further showed that NPR1 binding to SA requires the transition metal copper and is mediated through Cys^{521} and Cys^{529} of NPR1. These two residues are also required for the SA-induced expression of *PR1* in vivo. NPR1 was also capable of binding BTH. SA binding was shown to release the autoinhibitory effect of the BTB/POZ domain on NPR1 function, thus suggesting a conformational change in NPR1 resulting from SA binding, which likely also promotes disassembly of the NPR1 oligomers.

Recycling of the nuclear NPR1 protein by the proteasome pathway is critical for maximal expression of genes that are targets of NPR1 (Spoel et al. 2009). Recently, Fu et al. (2012) demonstrated that the NPR1 paralogues, NPR3 and NPR4, promote the SA-induced turnover of NPR1 by the proteasome pathway. NPR3 and NPR4 function as adaptors of the CUL3 (CULLIN3) ubiquitin E3 ligase to NPR1. In the absence of both, NPR3 and NPR4, the *npr3 npr4* double mutant accumulated elevated levels of NPR1 protein and exhibited enhanced basal resistance to the virulent pathogen *P. syringae* pv. *maculicola* ES4326. However, no further reduction in pathogen growth was observed in the systemic leaves of *npr3 npr4* plants that were previously inoculated on their lower leaves with an Avr pathogen. Fu et al. (2012) further demonstrated that NPR3 and NPR4 bind SA with different affinities in vitro. The K_d for NPR3 and NPR4 were 981 nM and 46.2 nM, respectively. SA promoted interaction between NPR3 and NPR1. However, it disrupted interaction between NPR4 and NPR1. It is suggested that in the absence of pathogen (i.e., low basal levels of SA) CUL3-NPR4-mediated degradation of

Fig. 2 (continued) phloem. G3P* and DIR1 have been suggested to facilitate long-distance transport of each other. DA* and G3P* promote accumulation of MES transcript (and likely the corresponding protein). Simultaneously, G3P* and DIR1 downregulate the expression of *BSMT1*, thus ensuring that the equilibrium is in favor of conversion of MeSA to SA. An amplification loop involving SA, the SA receptor NPR1, FMO1, and ICS1 promotes SA accumulation. NPR1 activation by SA leads to the expression of defense genes that contribute to SAR. *FMO1* is required for the induction of *ICS1* expression and accumulation of SA in the pathogen-free distal leaves. DA*, azelaic acid, and Pip signals converge at *FMO1*, which is required for activation of SAR by these signal molecules. It is likely that *FMO1* is also required for G3P*- and MeSA-induced SAR. However, this needs to be tested. *ALD1* is a point of convergence of the azelaic acid and Pip pathways. Pip acting through an amplification loop involving *FMO1* promotes *ALD1* expression and thus its own synthesis. DIR1 is essential for SAR induced by MeSA, G3P*, DA*, and azelaic acid. Whether it is required for Pip-induced SAR is not known. DA is shown to interact synergistically with azelaic acid and the *SFD1*-dependent mechanism. *White* and *gray boxes* represent the signaling molecules and biosynthetic enzymes, respectively. Signaling/transport proteins are represented by *black boxes/circles*. *Gray-filled arrows* represent long-distance transport. *Black arrows* ending in *black circles* indicate positive regulation (induction), while *black lines* ending with a *bar* indicate negative regulation. *Bidirectional arrows* indicate known synergistic interactions

NPR1 prevents spurious activation of defenses. In pathogen-inoculated plants, SA levels increase in the pathogen-bearing and systemic pathogen-free organs. The high levels of SA near the infection site result in the CUL3-NPR3-mediated turnover of NPR1, thus allowing cell death to be turned on. In the systemic tissues that have comparatively lower levels of SA, the turnover of NPR1 by CUL3-NPR3 is suggested to facilitate binding of newly synthesized NPR1 to the promoters of NPR1-regulated genes, thus promoting reinitiation of transcription at these promoters.

5 Concluding Remarks

The effects of SAR can be transmitted for a couple of generations (Luna et al. 2012). In addition, the manifestation of SAR confers a fitness advantage when plants are cultivated under disease pressure (Luna et al. 2012; Traw et al. 2007). However, SAR is an energy-driven process that requires diversion of resources from growth (Heidel et al. 2004; Pajerowska-Mukhtar et al. 2012). In addition, pathogen-derived effectors target genes and mechanisms that contribute to defense. The ability to recruit multiple signals empowers plants with better control over the activation of SAR. Several metabolites putatively involved in long-distance signaling have been described above. Figure 2 summarizes our current understanding of the potential interactions between these signal molecules during SAR. Progress also has begun to be made on understanding the mechanism of priming and the putative involvement of chromatin remodeling in SAR. The next 5 years will be important for unraveling the networking between these SAR signaling molecules and their liaison with priming and chromatin remodeling in SAR.

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References

Attaran E, Zeier TE, Griebel T, Zeier J (2009) Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in *Arabidopsis*. Plant Cell 21:954–971

Beckers GJ, Jaskiewicz M, Liu Y, Underwood WR, He SY, Zhang S, Conrath U (2009) Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in *Arabidopsis thaliana*. Plant Cell 21:944–953

Bohlmann J, Keeling CI (2008) Terpenoid biomaterials. Plant J 54:656–669

Chanda B, Xia Y, Mandal MK, Yu K, Sekine K-T, Gao Q-M, Selote D, Hu Y, Stromberg A, Navarre D, Kachroo A, Kachroo P (2011) Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. Nat Genet 43:421–427