Magical mystery tour: Salicylic acid signalling

Martin Janda a, b, Eric Ruelland c, d,*

a Department of Biochemistry and Microbiology, Institute of Chemical Technology Prague, Technické 5, 166 28 Prague 6, Czech Republic

b Laboratory of Pathological Plant Physiology, Institute of Experimental Botany AS CR, Rozvojová 313, 165 62 Prague 6, Czech Republic

c CNRS, UMR7618, Institut d’Ecologie et des Sciences de l’Environnement de Paris, 61 Avenue du General de Gaulle, 94010 Créteil, France

d Université Paris-Est Créteil, Institut d’Ecologie et des Sciences de l’Environnement de Paris, 61 Avenue du General de Gaulle, 94010 Créteil, France

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Salicylic acid (SA)
NPR1
SA overaccumulating mutants
Pathogen resistance
Phytohormones

ABSTRACT

Salicylic acid (SA) is a small phenolic compound whose therapeutic properties on human health have long been described. In plants, it is a key phytohormone that controls many physiological processes. In the last 20 years, great attention has been paid to its role in plant pathogen defence. The synthesis of SA is indeed one of the crucial ways a plant reacts to a biotic attack. SA is involved in both local and systemic resistance. SA metabolism (biosynthesis, conjugation and accumulation) and the signalling pathways that control SA levels are described here. The transcription factor NPR1 is an established cornerstone of SA signalling. Yet, both NPR1-dependent and NPR1-independent signalling pathways have been described, but very little is known about the effectors of the NPR1-independent pathway. We present work using different SA overaccumulating mutants as tools for studying the control of SA biosynthesis and the downstream effects of SA. Recently, new evidence has been provided concerning SA perception. Interestingly, NPR1 was proposed as a SA-receptor, as proteins involved in the control of NPR1 turnover. The different proteins involved in SA metabolism, in the regulation of SA levels and in the response to SA can define a “SA signalling module”. It is possible to use the genes encoding members of this module as indicators to identify stress situations where SA signalling is activated. This is illustrated for different biotic and abiotic stress responses.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Salicylic acid (SA), 2-hydroxy benzoic acid, is a phenolic compound named after the Latin name of the willow tree (Salix). Historically, willow bark has been used against fever and pain. In 1828, Johan Andreas Buchner isolated a yellow substance from willow bark, which he called “salicin”. A major breakthrough occurred in 1899, when the chemist Felix Hoffman, working for the Bayer company in Germany, synthetically made a derivative of SA, acetyl-salicylic acid, also known as aspirin, that has become the most used medical drug in the World (Vlot et al., 2009).

In plants, SA is a very important phytohormone. It regulates many physiological processes, such as cell growth, respiration, stomatal aperture, senescence, seed germination, seedling development and thermo-tolerance (Boatwright and Pajerowska-Mukhtar, 2013; Vlot et al., 2009). SA has a role in plant responses to many abiotic stresses such as chilling, heat, heavy metal toxicity, drought, osmotic stress and salinity (Horvath et al., 2007; Vicente and Plasencia, 2011). It also has a crucial role in plant pathogen response. The first mention of SA playing a role in plant defence was in 1979 (White, 1979). In 1990, two important studies described SA as a key component of Systemic Acquired Resistance (SAR); a mechanism by which plants gain a long-lasting protection against a broad spectrum of pathogens at sites distant from that of the initial pathogen attack (Malamy et al., 1999; Metraux et al., 1990). SAR is associated with the induction of Pathogenesis Related (PR) genes, which contribute to the resistance. PR genes are induced in both local and systemic tissues (non-infected tissue). Priming of plant defence conferred by SAR can persist for weeks to months, and possibly even over the whole growing season (Durrant and Dong, 2004).

Many actors playing a part in SA signalling mechanisms have since been deciphered (An and Mou, 2011; Fu and Dong, 2013; Vlot et al., 2009). In this review, we briefly highlight SA metabolism before focusing on how SA levels are regulated, with special attention being paid to the EDS1 node of upstream signalling from SA. We then describe the signalling events downstream from SA, including new evidence concerning SA perception.

Please cite this article in press as: Janda, M., Ruelland, E., Magical mystery tour: Salicylic acid signalling. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.07.003

http://dx.doi.org/10.1016/j.envexpbot.2014.07.003

0098-8472/© 2014 Elsevier B.V. All rights reserved.
2. Biosynthesis of SA and its conjugations

In plants, SA can be synthesized via two distinct enzymatic pathways, but to date neither have been fully described (Fig. 1). The first pathway involves Phenylalanine Ammonia Lyase (PAL; EC 4.3.1.5) that converts phenylalanine into trans-cinnamic acid (Vlot et al., 2009). PAL is encoded by four genes in Arabidopsis thaliana. In the second biosynthetic pathway, the key-enzyme is Isochorismate Synthase (ICS; EC 5.4.4.2) that catalyses the conversion of chorismate into isochorismate. ICS is encoded by two genes in Arabidopsis thaliana. This pathway has been shown to be the main SA biosynthetic pathway in response to pathogenic bacteria attacks, contributing to approximately 90% of total SA, with most ICS activity being due to ICS1, and ICS2 playing only a marginal role (Wildermuth et al., 2001). That said, the PAL pathway is also likely to contribute to pathogenic bacteria-related SA production since a pal1pal2pal3pal4 quadruple mutant exhibits a reduced amount of SA (both basal and after infection), and an increased susceptibility to Pseudomonas syringae (Huang et al., 2010). It was proposed that in the ICS-driven biosynthesis pathway, SA is synthesized in chloroplasts where both ICS1 and ICS2 are localized (Garcion et al., 2008; Strawn et al., 2007). Furthermore, when Pseudomonas putida NahG – a salicylhydroxylase (EC 1.14.13.1) that degrades SA to catechol – is ectopically expressed in plant chloroplasts, plants are unable to accumulate SA (Fragnere et al., 2011).

In plants, most of the SA synthesized is converted to SA O-glucoside (SAG) by SA glucosyltransferases (SAGTs; Fig. 1). Another, but less abundant, SA sugar-derivative is salicyloyl-glucose ester (SGE). SAGTs are localized in the cytosol and SA is actively transported into the vacuole where it is considered as an inactive storage form. Indeed, most conjugated SA forms are inactive. Another derivative of SA is methyl ester SA (MeSA). SA methyltransferase increases its volatility and membrane permeability, thus MeSA could serve as a long distance defence signal and it was proposed as one of the SAR mobile signals. Indeed, SA methyl transferase is required to produce the SAR signal in primary infected tissues, but not in systemic tissues (Dempsey and Klessig, 2012; Kumar and Klessig, 2008). SA can also be conjugated with amino acids, via the action of acyladenylate/thioester-forming enzyme (GH3.5), which is involved also in the conjugation of amino acids to the auxin. SA can be metabolized through hydroxylation by Salicylate–3-hydroxylase (SH3) to 2,3-dihydroxybenzoic acid (2,3-DHBA; gentisic acid) (Ibrahim and Towers, 1959). SH3 transcription is induced in response to an increase in SA level and therefore, SH3-dependent hydroxylation could serve to prevent SA over-accumulation (Zhang et al., 2013). Finally, sulfonation of SA has been proposed since SA is a substrate of sulfotransferases in vitro, however there is no in vivo evidence, to date (Baek et al., 2010; Dempsey et al., 2011; Vlot et al., 2009).

3. Control of SA biosynthesis during a pathogen attack

The signalling mechanism controlling the level of SA upon a pathogen attack has been studied intensively during the last 20 years. SA signalling pathway can be triggered both by pathogen triggered immunity (PTI) and effector triggered immunity (ETI) (Mishina and Zeier, 2007). Indeed, in plants, these two modes of plant immunity are defined according to the way pathogens are detected. During PTI, pattern recognition receptors (PRRs) recognize molecular patterns that are relatively conserved among similar types of microbes, the so-called microbe-associated molecular patterns (MAMPs) (Kim et al., 2014). On the other hand, ETI is triggered by the specific recognition of effectors by resistance (R) proteins, which are often nucleotide-binding leucine-rich repeat (NB-LRR) proteins (Tsuda et al., 2013). From an evolutionary point of view, considering plant and pathogen co-evolution, PTI is considered as the first layer of immunity that allows plants to recognize pathogen molecular patterns (e.g. flagellin, elongation factor, chitin). Later in evolution, pathogens started to release effectors into plants to suppress PTI. To counteract this, evolution selected plants with specific R-proteins that interact with specific effectors thus triggering ETI and, in most cases, leading to plant cell death (Jones and Dangl, 2006). Both PTI and ETI lead to enhanced SA production via the ICS biosynthetic pathway (Wildermuth et al., 2001). Several actors controlling the regulation of SA accumulation during a pathogen attack have been identified.

SAR deficient 1 (SARD1) and Calmodulin Binding Protein 60g (CBP60g) are both transcription factors (TFs) critical for the regulation of ICS expression (Wang et al., 2011a). In a sard1 cbp60g double mutant, the synthesis of SA induced by ETI (or UVB light) is blocked and both SAR and basal defences are compromised. Both SARD1 and CBP60g can bind to the ICS1 promoter at its GAAATTTGG sequence (Fu and Dong, 2013; Wang et al., 2011a). An important compound for SA biosynthesis and signalling is EDSS (Enhanced Disease Susceptibility 5) a member of the MATE (multidrug and toxic compound extrusion) family of transporters, localized to the envelope membrane of chloroplasts (Yamasaki et al., 2005). EDSS has been proposed to transport SA from chloroplasts to the cytosol (Serrano et al., 2013). The eds5 mutant accumulates low amounts of SA and shows a highly reduced PR-1 expression after pathogen infection. Consequently, eds5 plants are hyper-susceptible to pathogens (Nawrath et al., 2002).

4. The EDS1 PAD4 SAG101 triad

Enhanced Disease Susceptibility 1 (EDS1), Phytoalexin Deficient 4 (PAD4) and Senescence Associated Gene 101 (SAG101) are components of the plant signalling machinery controlling SA synthesis in response to pathogens. EDS1 interacts with either PAD4 or SAG101 to form hetero-complexes that are necessary for SA signalling. After a virulent pathogen attack (Pseudomonas syringae pv tomato), ICS1 is not induced in eds1 or pad4 mutants, and SA is not accumulated (Feyts et al., 2001; Garcia et al., 2010). The exogenous application of SA can rescue defence gene induction in these mutants (Falk et al., 1999; Feyts et al., 2001; Zhou et al., 1998). EDS1 is recruited by TIR-NB-LRR receptors (Toll Interleukin Receptor-Nucleotide Binding Leucin Rich Repeat, TNLs) during ETI. Indeed, the action of RPS4 (Resistance to Pseudomonas syringae 4), a typical TIR-NBS-LRR, requires EDS1 to induce SA-dependent and SA-independent resistance to pathogens (Feyts et al., 2005; Garcia et al., 2010; Wiemer et al., 2005; Wirthmueller et al., 2007). How EDS1 acts to control SA synthesis is not completely understood. EDS1, PAD4 and SAG101 all contain a conserved lipase-like domain, but the catalytic function of this domain is not required for EDS1 responses, ETI and basal resistance.
However, the lipase-like domain of these three proteins is required to promote interactions between EDS1 and PAD4 or EDS1 and SAG101 (Rietz et al., 2011; Wagner et al., 2013). For the complete plant innate immune response, there is evidence that the balanced cytoplasmic and nuclear activities of EDS1 are necessary (Garcia et al., 2010). An important role in this process is played by nuclelopores, namely Nucleoloparin8/MOS7 (Cheng et al., 2009). Because SA treatment induces EDS1 and PAD4 transcription in WT plants, it was postulated that PAD4 and EDS1 work in a positive feedback loop regulated by SA. Indeed, SA treatment in WT plants induces EDS1 and PAD4 transcription (Falk et al., 1999; Feyes et al., 2001; Vlot et al., 2009; Zhou et al., 1998). When ETI recognition is via CC-NBS-LRR receptors (Coiled Coil Receptor-Nucleotide Binding Site-Leucin Rich Repeat, CNLs), Non–Race Specific Disease Resistance 1 (NDR1) contributes to SA synthesis (Bernoux et al., 2011). NDR1 is a glycosphatidylinositol-anchored plasma membrane protein and impairment of NDR1 function suppresses defence reactions after PTI or ETI while the over-expression of NDR1 reduces the growth of virulent bacteria (Coppering et al., 2004; Shapiro and Zhang, 2001; Vlot et al., 2009).

5. SA-overaccumulating mutants give an insight into the complexity of the control of SA level

Over the past two decades, many mutants that constitutively accumulate SA have been identified. We have established a list of twenty-three SA-overaccumulating mutant genotypes for which the protein function affected by the mutation is known (Table 1). We were interested in comparing PR-1 transcription, growth and pathogen resistance properties in these mutants. Based on their altered properties, they could be divided into two classes: loss of function (LF) or gain of function (GF) mutants (Table 1). The LF mutants are mutated in proteins that negatively control the basal level of SA while the GF mutations affect proteins that positively control SA level. In control conditions, all of these mutants exhibit a higher SA content compared to WT plants and they all constitutively express PR-1. Twenty of them are described as having a dwarf stature while eighteen of them exhibit an enhanced resistance to pathogens. Interestingly, eight proteins affected by mutations are connected with lipid metabolism, namely acd5, acd11, bap1, fah1/fah2, gonst1, pik4/kfj2, sncl and ssi2. It suggests a close connection between SA signalling and lipid signalling (Table 1) (Janda et al., 2013).

Recently, experimental evidences showed a connection between SA and sphingolipids. Sphingolipids are constructed on the condensation of a serine with a fatty acid. The result of this condensation is the so-called long chain bases. Depending on chemical modifications we can distinguish amongst free long-chain bases phytosphingosine, sphingamine and sphingosine (amongst others). Long-chain bases can further be acylated, resulting in ceramides. Ceramides can be further glucosylated, leading to glucosylceramides, and glycosylcolinosphosphorylcercamids (complex sphingolipids) (Konig et al., 2012). Four SA over-accumulating mutants from Table 1 are altered in the sphingolipid metabolism (acd5, acd11, fah1/fah2, gonst1). Indeed, acd5 is mutated in ceramidine kinase; this mutant over accumulates the kinase substrates, i.e. ceramides (Liang et al., 2003). ACDD11, is a Ceramide–1–Phosphate Transfer Protein. An overall accumulation of total sphingolipids (including long-chain bases) is evidenced in acd11 compared to wild type background, with total free ceramides showing the greatest accumulation (Simanshu et al., 2014). The fah1/fah2 double mutant is impaired in Fatty Acid α-Hydroxylase. It exhibits reduced amount of sphingolipids with α-hydroxylated fatty acid moieties; conversely it has increased amount of sphingolipids without these moieties. Besides, in fah1/fah2 double mutant, long-chain bases and ceramides are overaccumulated but level of complex sphingolipids is reduced (Konig et al., 2012). Golgi–Localized Nucleotide Sugar Transporter 1 (GNOST1) was shown in vitro to be able to transport all GDP-D-sugars. In the gonst1 mutant, the glycosylation of sphingolipids is altered, thus leading to less complex sphingolipids containing hexoses in their sugar moiety (Martinier et al., 2013). As we mentioned all these mutants contain high level of SA. In addition Wang et al. (2008) showed that the erh1 mutant is mutated in Inositolphosphorylcercamide Synthase (IPCS). This mutant leads to SA overaccumulation, dwarf phenotype and enhanced resistance to Golovinomyces cichoracearum but not to Pseudomonas syringae pv maculicola. The erh1 mutant was not included into Table 1 because the background in which it was observed is not Col-0 but is Col-0 expressing R-gene RPW8 under native promoter. The loss of function of ERH1 results in higher levels of ceramides (Wang et al., 2008). Rivas–San Vicente et al. (2013) showed that Nicotiana benthamiana transgenic plants with silenced for the gene encoding the LCB2 subunit of heterodimeric enzyme Serine Palmitoyltransferase (SPT) had higher SA amount and increased PR-1 transcription. In transgenic plants, the different long chain bases were differentially altered with total levels of phytosphingosine decreasing and that of sphingamine and sphingosine increasing (Rivas-San Vicente et al., 2013). Taken together, these data suggests higher amounts of long chain bases and ceramides triggers SA biosynthesis. But still a lot of about connection of sphingolipids and SA is hidden and deeper investigation is needed for understanding of this intriguing connection.

In selected mutants, we were particularly interested in the dependency of growth, SA accumulation, PR-1 expression and resistance on EDS1, PAD4, ICS1 and SA (based on NahG experiments) (Fig. 2). The acd11, pik4/kfj2 and snc1 mutants exhibit similar properties. Their stunted growth and PR-1 expression are dependent on high amounts of SA that are dependent on ICS1 and EDS1. But interestingly, older sid2ac11 double mutant plants revert back to an acd11 phenotype. In snc1, the resistance to P. syringae pv maculicaola is dependent on EDS1 and PAD4 (Šašek et al., 2014; Brodersen et al., 2002, 2005; Li et al., 2001; Zhang et al., 2003). In ssi2, the situation is complicated because high SA levels and PR-1 expression are ICS1-dependent but EDS1-independent. However, the stunted growth is both EDS1- and ICS1-independent and this is reverted to the WT size in the sid2 ed1 ssi2 triple mutant (Venugopal et al., 2009). In snc, growth is EDS1-independent, and PR-1 expression and resistance to H. arabidopsis is partially dependent on EDS1 (Bi et al., 2010). Cpr1 and sr1 (also called camt3) exhibit similar properties; SA accumulation, growth, PR-1 expression, resistance to P. parasitica (for cpr1) and to P. syringae pv tomato (for sr1) are PAD4 dependent (Clarke et al., 2001; Du et al., 2009; Jurage et al., 2001; Qiu et al., 2012). In dnd1 and dnd2 mutants, SA accumulation and growth are PAD4 independent while the resistance to P. syringae pv maculicaola is PAD4 dependent (Jurage et al., 2001). In ssi4 mutants, PR-1 expression and growth are EDS1-dependent. Interestingly, based on experiments with the NahG ssi4 double mutant, growth is only partially dependent on SA (Shirano et al., 2002) (Fig. 2).

The intriguing question of how SA is involved in the interconnection between pathogen resistance and growth is still poorly understood but a better characterization of SA over-accumulating mutants and their crossed mutants could serve as important tools to help decipher these interconnections.

6. Molecular signalling components downstream from SA

6.1. NPR1-dependent signalling

The accumulation of SA is associated with the induction of PR genes. In 1994, Cao et al. searched for mutants that did not express...
Fig. 2. Schematic overview of growth and PR-1 expression dependency of selected SA overaccumulating mutants with respect to either EDS1 or PAD4. When data are available from crossings with sid2 (ICS1) and/or NahG (SA) they are incorporated in the pathways. If data from crossing with sid2 and NahG missing, the high SA is on the same level as PR-1 expression and/or growth. The grey crossed arrows represent cases in which direct evidence shows that PR-1 expression and/or growth are independent on EDS1/PAD4. A–D represents dependency on EDS1, E–F represents dependency on PAD4. (A) acd11, p4kiβ1β2, snc1, (B) ssi2, (C) snc4, (D) ssi4, (E) dnd1, dnd2, (F) cpr1, sr1.

Fig. 3. Model of SA downstream signalling. Elevated SA levels lead to NPR1 monomerization of and the translocation of NPR1 monomers to the nucleus where they induce defence gene expression. NPR3/NPR4 controls NPR1 degradation in the nucleus by the proteasome. SA – salicylic acid; NPR1/3/4 – nonexpressor of Pathogenesis Related 1/3/4; as-1 – associated sequence 1; TGA – transcription factors; TRX – thioredoxin; GSNO – nitrosoglutathione; MOS – modifiers of snc1; ETI – effector triggered immunity; SAR – systemic acquired resistance.
Table 1
SA overaccumulating mutants.

<table>
<thead>
<tr>
<th>Mutant</th>
<th>PR-1 Mutation</th>
<th>Growth</th>
<th>Resistance</th>
<th>Protein</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>NPR1</td>
<td>npr1</td>
<td>NPR1</td>
<td>npr1</td>
<td>NPR1</td>
</tr>
<tr>
<td>acd5</td>
<td>LF</td>
<td>(++)</td>
<td>ND</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>acd11</td>
<td>LF</td>
<td>(++)</td>
<td>WT</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>bap1</td>
<td>LF</td>
<td>(++)</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>bir1</td>
<td>LF</td>
<td>(++)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>bon1</td>
<td>LF</td>
<td>(++)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>cpr1</td>
<td>LF</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>dnd1</td>
<td>LF</td>
<td>(++)</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>dnd2</td>
<td>LF</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>jah1/fah2</td>
<td>LF</td>
<td>(++)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>gnos1</td>
<td>LF</td>
<td>(++)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>mpk4</td>
<td>LF</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>rop6</td>
<td>LF</td>
<td>(++)</td>
<td>WT</td>
<td>(U)</td>
<td>(U)</td>
</tr>
<tr>
<td>pikb/bj1/bj2</td>
<td>LF</td>
<td>(++)</td>
<td>WT</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>sfr1</td>
<td>LF</td>
<td>(++)</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>snr1</td>
<td>LF</td>
<td>(++)</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>sr1</td>
<td>LF</td>
<td>(++)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ssi2</td>
<td>LF</td>
<td>(++)</td>
<td>(++)</td>
<td>(−)</td>
<td>WT</td>
</tr>
<tr>
<td>sncl</td>
<td>GF</td>
<td>(++)</td>
<td>WT</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>sn2</td>
<td>GF</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>sn4</td>
<td>GF</td>
<td>(++)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>acd6</td>
<td>GF</td>
<td>(++)</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>ssi4</td>
<td>GF</td>
<td>(++)</td>
<td>(++)</td>
<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>dll1</td>
<td>GF</td>
<td>(++)</td>
<td>WT</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

GF – gain of function mutation; LF – loss of function mutation; ? – not known; (a) Pseudomonas syringae pv maculicola; (b) Pseudomonas syringae pv tomato; (c) Botrytis cinerea; (d) Golinivovymyces orontii; (e) Golinivovymyces choloraeforum; “induction of PR-2, expression visible only in local leaves; ++ as a WT but exhibits lesions; R – resistance; S – susceptible; NC – no change, ND – not determined [based on our knowledge]; (+++) – PR-1 induction corresponding to particular single mutant; WT – the similar pattern as in wild type plants; (+) – compromise in compare to single mutant but higher than WT; (−) – growth corresponding to particular single mutant; (−−) – even smaller than single mutant; (++) growth partly reversed; (U) – unique (curved leaves, stunted height); acd1, acd6, acd13 (accelerated cell death); cpr1, cpr2, cpr3, cpr4, cpr5 (constitutive expression of PR genes); ssi1, ssi2, ssi3, ssi4 (polysaccharide insensitive); snr1, snr2 (suppressor of npr1–1, inducible); dnd1, dnd2, dnd3 (defense no death); mpk4 (MAP kinase 4); bon1, bon5 (bon1, bna1, bna2, bna3, bna4, bna5 (bon1-associated protein 1); fah1 (fatty acid hydroxylase); gnos1 (golglocalized nucleotide sugar transporter 1); sr1 (signal responsive 1) also called camit (calmodulin-binding transcription activator 3); pikb/bj1/bj2 (phosphatidylinositol-4-kinase); sncl, sn2, sn3, sn4 (suppressor of npr1–1, constitutive); and dll1 (disease like lesion).

**PR genes in the presence of exogenous SA. They found and characterized npr1, a mutant that does not exhibit PR-2 transcription. Consequently, the Nonexpressor of Pathogenesis Related 1 protein (NPR1) was established as a key component of SA signalling (Cao et al. 1994). NPR1 was shown to be a central transcriptional regulator controlling the expression of most SA-dependent genes (Wang et al. 2005). NPR1 is present as an oligomer in the cytosol but monomerizes upon a SA level increase (Fig. 3). The effect of SA on NPR1 monomerization arises from SA-triggered changes in cell redox potential (see below). The Cys82 and Cys216 are important for oligomeric structure of NPR1 (Mou et al. 2003). The SA-triggered decrease in redox status leads to the reduction of this bond, most likely via the action of a cysteic thiorodoxin such as TRXh5 and/or TRXh3. On the other hand, the S-nitrosylation of NPR1 by S-nitrosoglutathione (GSNO) facilitates its oligomerization. The Cys concerned in this process has been identified as Cys156 (Tada et al. 2008). NPR1 monomers translocate to the nucleus where they activate the transcription of defence-related genes. It was suggested that MOS6, a homologue of nucleoprotein 96, MOS6 (importin a3) and MOS7, the homologue of nucleoprotein 88, are involved in the NPR1 nuclear translocation process (Vlot et al. 2009). The mos3, mos6 and mos7 single mutants are impaired in the activation of defence responses induced by SA (Cheng et al. 2009; Palma et al. 2005; Zhang and Li 2005). The localization of NPR1–GFP in the mos7 mutant clearly established a role for MOS7 in NPR1 nuclear accumulation (Cheng et al. 2009). In the nucleus, NPR1 monomers bind to TGA transcription factors and this is followed by their direct binding to an as–l (activation sequence 1) cis-regulatory element that is present in the promoters of PR genes, thus activating their expression (Jakoby et al. 2002). In particular, TGA2, 5, and 6 are required for SA-induced PR-1 expression (Mou et al. 2003). Interestingly, an npr1 mutant is characterized by a higher SA level after infection compared to WT plants (DeFraia et al. 2010). When NPR1 is unable to localize to the nucleus, ICS1 over-accumulates and a toxic SA level occurs. This indicates that NPR1 acts as a negative regulator of SA biosynthesis and ICS1 expression (Zhang et al. 2010).**
NPR1 is submitted to phosphorylation and this promotes its degradation by the 26S proteasome due to polyubiquitination by Cullin3 (CUL3) [Spenol et al., 2009] (Fig. 3). Recent work provides evidence that NPR1-signalling is far from linear. A novel transcription factor, TL1-binding factor 1 (TBF1), is required for the induction of NPR1-dependent gene expression [Pajarowska-Mukhtar et al., 2012]. TBF1 regulates the expression of ER-resident genes. The ER-resident genes are induced by SA coordinately with PR gene expression and they are responsible for the secretion of PR proteins. In a tbf1 mutant, SAR and PTI are both compromised. Upon SA treatment, TBF1 also down-regulates genes that encode proteins with a chloroplastic function. Finally, at the transcript level, NPR1 and TBF1 act in a positive feedback loop, regulating the transcription of each other. How these two “master” regulators cooperate require further investigation (Fu and Dong, 2013; Pajarowska-Mukhtar et al., 2012; Wang et al., 2005).

6.2. NPR1-independent signalling

Much evidence indicates the existence of a SA-dependent but NPR1-independent signalling pathway. Transgenic plants expressing a bacterial NahG gene (encoding salicylate hydroxylase), in which SA is metabolized to catechol, show a suppressed ETI, thus suggesting that SA is necessary for this response. However, a suppressed ETI is not observed in an npr1 mutant [Rairdan and Delaney, 2002; Shah et al., 2001]. This observation suggests that certain SA-dependent responses can be NPR1-independent. Most information concerning an NPR1-independent signalling pathway comes from studies of SA-overaccumulating mutants. Indeed, these mutants are interesting tools to study the effect of endogenous SA. As already mentioned, such mutants constitutively express PR genes, including PR-1 (Table 1). When some of these mutants are crossed with npr1, the resulting mutants can exhibit normal WT PR-1 expression levels. In these mutants, the high SA signal is transmitted through the NPR1 dependent pathway. This is the case of sncl, acad11, ddi1 and rop6 mutants. However, by crossing some of these mutants with npr1 results in plants showing either a compromised or an unaffected PR-1 transcription. In this way, PR-1 expression appears to be independent of NPR1 in sncl2, ssi2, ssi4 and snl1 mutants while PR-1 expression is only partially dependent on NPR1 in acad6, dnd1, dnd2 and p4kpb1 mutants when compared to WT plants. In these mutants, both NPR1-dependent and NPR1-independent pathways participate in PR-1 expression.

As already mentioned, in plants overaccumulating SA, an effect of the high SA levels is to induce a stunted stature. Therefore, it is possible to examine whether mutants exhibiting high SA levels still have a dwarf phenotype when they are crossed with npr1 (Table 1). The stunted stature trait appears to be NPR1-independent in sncl, sncl2, snl1, dnd1, dnd2 and rop6. We can note the unique feature of cp1 and ssi4 for which the crossing with npr1 leads to even smaller plants. An observed incomplete reversion suggests the trait is only partially dependent on NPR1 in the acad11 mutant. A clear and total (or nearly total) stature reversion was observed in three mutants, acad5, ssi2, p4kpb1. For these mutants, the stunted stature appears to be strictly NPR1-dependent. In four mutants, NPR1 dependency is different with respect to PR-1 expression and growth: in sncl and acad11, PR-1 expression is NPR1-dependent while growth is independent (partially dependent in acad11). Interestingly, the growth is NPR1-dependent in p4kpb1 but PR-1 expression is decreased in p4kpb12npr1 although it remains higher compared to the WT. A similar situation to p4kpb12 is seen for ssi2 (Table 1).

The situation with respect to pathogen resistance is also interesting since many of the mutants exhibit differences between NPR1 and npr1 backgrounds. The resistance to oomycetes (Peronospora parasitica/Hyaloperonospora arabidopsis) seems to be NPR1-independent in all of the SA-overaccumulating mutants studied so far, except for the dnd1, dnd2 and bap1 mutants. The resistance to P. syringae pv tomato is NPR1-dependent in the sncl1, ssi2 and bap1 mutants, and to P. syringae pv maculicola in the cpr1 and ssi2 mutants. NPR1-independent resistance to P. syringae pv maculicola is described in both sncl and acad6 and to P. syringae pv tomato in dnd1, dnd2 and mpk4. It should be noted that in most cases, the resistance to only a single Pseudomonas pathovar has been determined (Table 1).

Further evidence for SA-induced NPR1-independent changes include the Atwhy1 mutant that exhibits abolished PR-1 expression; the AtWHY1 transcription factor binds to DNA in a SA-dependent and NPR1-independent manner. AtMYB30 is another SA-induced transcription factor that positively regulates plant response to pathogen in SA dependent and NPR1 independent manner (Desveaux et al., 2004, 2005; Raffaele et al., 2006).

It is clear from the work carried out using SA overaccumulating mutants that one or several NPR1-independent pathways exist and they play very important roles in plant pathogen resistance. However, the NPR1-dependent pathway is the major route in SA signalling but the use of alternative pathways when NPR1 is missing is a nice example of the robustness of plant defence signalling.

7. SA perception

How SA is perceived still holds some mystery even though several SA-binding proteins (SABPs) have been identified; methyl salicylate esterase, catalase, cytosolic ascorbate peroxidase and chloroplastic carbonic anhydrase. Methyl salicylate esterase (also called SAPBP) has the highest affinity for SA compared to the others (Du and Klessig, 1997) while MeSA is the in planta substrate (Park et al., 2007). The proposed role of SAPBP is to release SA from MeSA in systemic tissues (Kumar and Klessig, 2008) and therefore it cannot be considered as a SA receptor. SA inhibits the H2O2-scavenging activities of catalase and ascorbate peroxidase and by this mechanism, SA facilitates H2O2 accumulation induced by avirulent pathogens (Fu et al., 2012; Vlot et al., 2009). Catalase and ascorbate peroxidase could be considered as SA receptors but they are also SA effectors contributing to SA action via cell metabolism. In 2011, Maier et al. reported that NPR1 was sensitive to SA treatment because SA diminished the binding between NPR1 and NIMIN2 and NIMIN1, while it did not affect the binding of NPR1 to TGA in yeast two hybrid assays (Maier et al., 2011). These observations might suggest that NPR1 can bind SA. Indeed, using a special equilibrium dialysis ligand binding method, it was shown that NPR1 is a SA binding protein. The inactive structural analogues of SA do not bind NPR1. (Wu et al., 2012) The affinity of NPR1 to SA is similar to that of other hormone receptor interactions. The binding to SA might cause a conformational change that releases the C-terminal transactivation domain from an N-terminal autoinhibitory domain. The binding to SA therefore allows the transcriptional activity of NPR1. Besides this role, the binding of SA might be necessary also for the monomerization of NPR1 oligomers brought about in reducing conditions. NPR1 thus appears to be a SA receptor, but also an effector for PR gene expression. Interestingly it was also found that SA binds to two NPR3 paralogs, NPR3 and NPR4. The binding of SA to NPR3 occurs with a lower affinity when compared to NPR4. Their role in SA signalling is connected with NPR1 turnover. NPR3 and NPR4 serve as Cullin 3 (CUL3) E3 ubiquitin ligase adapters to promote the proteasomal degradation of NPR1. The role of NPR3 and NPR4 in promoting NPR1 protein degradation, together with the differences in SA affinity towards NPR3 and NPR4 explains many aspects of NPR1 homeostasis at the site of infection, in distal tissues and with respect to basal resistance (see Fig. 3). NPR1 interacts with SA-bound NPR3 and unliganded NPR4. Upon infection, the concentration of SA increases at the site of infection. This very
high SA concentration promotes the interaction between NPR1 and SA-bound NPR3 thereby leading to the proteasomal degradation of NPR1 and thus promoting local cell death. In the distal site of infection, a moderately high concentration of SA occurs (higher than the basal concentration but not as high as at the infection site) and the NPR1-NPR3 interaction is reduced, allowing some NPR1 to enter the nucleus to promote PR-1 transcription. In the absence of infection, the very low basal SA concentration favours the interaction of NPR1 with unliganded NPR4 while some NPR1 escapes degradation, thus allowing limited NPR1-dependent expression of defence genes to confer basal resistance (Fu et al., 2012; Kaltdorf and Naseem, 2013).

8. SA transduction pathways

8.1. Phospholipid signalling and SA

In Arabidopsis suspension cells, SA stimulation leads to the rapid activation of Phospholipase D (PLD, EC 3.1.4.4) (Krinke et al., 2009). The PLD enzyme activity leads to creation of important signalling molecule phosphatidic acid (PA). Treatment with SA increases either the PA level or the PLD activity in Arabidopsis thaliana, Brassica napus and Glycine max (Kalachova et al., 2012; Profotova et al., 2006; Rainteu et al., 2012). In the presence of primary alcohols, that quench PA formation by PLDs, gene expression upon SA addition is strongly affected, showing the importance of PLD activity in the control of SA-triggered transcriptome changes (Krinke et al., 2009). Amongst these PLD-dependent genes is PR-1. Interestingly, in Arabidopsis suspension cells, SA also activates a Type-III Phosphatidylinositol-4-Kinase (PI4K, EC 2.7.1.67) leading to the formation of phosphatidylinositol-4-phosphate (PI4P) and phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2) (Krinke et al., 2007). A considerable overlap between PLD- and PI4K-controlled genes has been observed. Amongst the genes controlled similarly by both pathways is PR-1. This led to the idea that SA activates PI4K, increasing PI4P and PI-4,5-P2, with the latter lipid activating some PLD isoforms. NPR1 induction by SA is abolished by including n-butanol but not by inhibiting type-III PI4K, suggesting that in response to SA, both PI-4,5-P2-dependent PLDs and PI-4,5-P2-independent PLDs are brought into play (Krinke et al., 2007, 2009). Identifying the PLDs activated in response to SA will help validate this hypothesis. In Arabidopsis, the expression of PI-4,5-P2-dependent PLDs, PLDJ1.2 and PLDy1 and PLDy3s, are induced by SA (Janda et al., 2013; Zhao et al., 2013). This observation might indicate that they have a role in SA signal transduction.

PI-PLC could also have a role in SA signal transduction in plants. Indeed, the observed increase of PI-4,5-P2 in suspension cells could result from coconcomitant in vivo PI4K activation and PI-PLC inactivation by SA. Interestingly, in vitro enzymatic assays of microsomes extracted from Capsicum chinense Jacq. suspension cells, 30 min after SA treatment, showed that SA provoked an increase in lipid kinase activities leading to the production of PI4P and PI-4,5-P2 and a decrease in PI-PLC activity (Altuzar-Molina et al., 2011). This fits well with what is observed in vivo in Arabidopsis suspension cells. The very short period in which these in vitro changes occur after SA treatment most likely implies that PI4K and/or PI-PLC are subject to posttranslational modifications but this has to be confirmed by further investigations.

8.2. Protein phosphorylation

Protein phosphorylation is a common posttranslational modification used in plant signalling. Although very few data document a possible role of protein phosphorylation downstream from SA, it might be of some importance. Okadaic acid, a protein phosphatase inhibitor, suppresses PR-1 expression in the presence of SA (Conrath et al., 1997), thus implying that the phosphorylation balance of some protein might be involved in SA signal transduction. In tobacco suspension cells, SA induces a rapid and transient activation of a 48 kDa protein kinase called SIPK (for SA-Induced Protein kinase) that is orthologous to AtMPK6 (Vlot et al., 2009; Zhang and Klessig, 1997). Analysis of transgenic tobacco BY2 suspension cells revealed that SIPK: GFP was both cytosolic and nuclear. SIPK phosphorylates the tobacco WRKY1 transcription factor (Menke et al., 2005). Indeed, important phosphorylation targets in response to SA are proteins of the transcription machinery. SA induces a Casein Kinase II-like (CK2-like) activity that phosphorylates the NPR1-interacting TGA2 transcription factor. SA treatment enhances this interaction (Kang and Klessig, 2005). Nuclear-located NPR1 is phosphorylated on Ser11 and Ser15 in response to in vivo SA treatment. This SA-induced phosphorylation of NPR1 facilitates its interaction with the CUL3-based ubiquitin ligase and stimulates turnover (Spoel et al., 2009).

8.3. Redox status and reactive oxygen species (ROS)

SA signalling is connected to cell redox status. Indeed, NPR1 is monomerized via the reduction of disulphide bridges. The redox status of the cell, as already mentioned, is controlled by glutathione levels and the balance between their oxidized (GSSG) and reduced (GSH) forms. A 15:1 GSH/GSSG ratio leads to NPR1 monomerization, whereas a ratio lower than 7.5:1 triggers oxidation of the monomeric form and the subsequent production of the oligomeric form (Mou et al., 2003). 2,6-Dichloro-isonicotinic acid (INA) is a SA homologue that causes the monomerization of NPR1 and its translocation into the nucleus. Treatment with INA causes a dramatic decrease of both GSH+GSSG content and GSH/GSSG ratio after 8 h and this is followed by a sharp increase that reaches a plateau after 24 h. This suggests a biphasic redox response to SA or SA homologues. In plants inoculated with the bacterial pathogen Pseudomonas syringae pv maculicola ES4326 carrying the avirulence gene,avrRpt2, total glutathione and the GSH/GSSG ratio were both significantly increased in the uninfected distal tissues, 24 h after infection. It is likely that this reduction of the glutathione pool is SA-mediated (Mou et al., 2003). Indeed, direct application of SA caused the increase of GSH+GSSG and GSH/GSSG after 8 h (Spoel and Loake, 2011). Interestingly SA binds and inhibits the glutathione–transferase activities of GSTF8, GSTF10 and GSTF11. Binding of SA to GSFT could contribute to modulating glutathione homeostasis, and therefore redox cell status since this is indeed largely controlled by glutathione concentration and the ratio between its reduced and oxidized forms (Tian et al., 2012).

It was stated by Vlot and co-workers that “The relationship between SA and ROS is complicated” (Vlot et al., 2009). It has been proposed that SA and ROS function in self-amplifying feedback loops in response to stress conditions so that a high SA level brings about a high H2O2 content (Chen et al., 1993; Fauth et al., 1996; Rao et al., 1997). On the other hand, treatment with H2O2 causes the accumulation of SA (Leon et al., 1995). These observations lead to the situation where we still do not know if SA is upstream or downstream of H2O2.

8.4. Nitric oxide (NO)

Another signalling molecule interacting with SA signalling is NO. SA accumulation is induced by NO donors while NO effects on pathogen defence require SA (Grun et al., 2006; Wendehenne et al., 2004). For instance, in the NahG-expressing mutant, NO-induced local and systemic resistance in TMV-infected tobacco is suppressed, thus showing that NO effects require SA (Song and Goodman, 2001). Therefore, NO might act via the SA signalling pathway. The mode of action of NO is still unclear, but a possible
target could be NPR1 through N-nitrosylation, which leads to the stabilization of oligomeric NPR1 forms (Tada et al., 2008).

8.5. Actin cytoskeleton

Treatment of tobacco plants with cytochalasin E, an inhibitor of actin microfilament polymerization, leads to PR-1 expression (Kobayashi and Kobayashi, 2007). Recently, we have shown that the transcription of PR-1 and PR-2 is induced by nanomolar concentrations of latrunculin B, an actin depolymerizing drug, and micromolar amounts of cytochalasin E. This induction already occurs 6 h after the treatment of Arabidopsis thaliana seedlings and expression continues to increase up to 24 h (Matouskova et al., 2014). It was shown also that treatment with SA leads to disruption of actin filaments, resembling the pattern observed after a treatment with 200 nM latrunculin B (Matouskova et al., 2014).

Therefore, depolymerization of actin can mimic SA treatment with respect to PR genes induction.

9. The SA signalling module and its use to identify conditions where SA is activated

So far in this review we have described major components of SA signalling, including proteins and genes controlling SA level in response to pathogens, and proteins involved in SA signal transduction and the control of SA-triggered responses. These components make up what we could call the “SA signalling module”. This module is composed of 14 genes connected with SA signalling. Most of these genes encode proteins that participate either upstream of SA production or in SA signal transduction, and they have been described above. SA-induced genes, such as PR-1, PR-2, and PR-5 (Clarke et al., 2000), and the transcription factor WRKY38

Please cite this article in press as: Janda, M., Rueland, E., Magical mystery tour: Salicylic acid signalling. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.07.003
Hyaloperonospora cichoracearum gene (bacteria, fungi, oomycetes): Pseudomonas syringae in different pathogens (maculicola, phaseolicola, tomato), Botrytis cinerea and Plectosphaerella cucumerina, Phytophthora infestans, Blumeria graminis, Golovinomyces orontii, Golovinomyces cichoracearum, Hyaloperonospora Arabidopsis (Perenospora parasitica), Phytophthora parasitica and Fusarium oxysporum (Berrocal-Lobo and Molina, 2004; Both et al., 2005; Coates and Beynon, 2010; Fabro et al., 2008; Glazebrook, 2003; Gunwald and Flier, 2005; Micali et al., 2011; Thomma et al., 2000; Wang et al., 2011b; Xin and He, 2013). Microarray experiments of plants treated with SA or benzothiadizol (BTH), a functional analogue of SA, have been included as positive controls. Obviously, most pathogens induce transcription of SA-related genes. However, the powdery mildew fungi G. Cichoracearum induces a very interesting pattern of gene response; at earlier times after treatment (18, 36 hpi), “SA signalling module” gene expression is suppressed, whereas from 72 hpi these genes become strongly induced. This suggests that the plant changes its defence response during this time period (Fig. 4).

SA signalling is not activated only by biotic stresses. The same set of genes was used to examine stress conditions that affect their transcription. Again by mining Genevestigator microarray data with respect to our “SA signalling module” gene list (Fig. 4), we were able to identify stresses known to trigger SA accumulation (ozone, UV) and also gamma irradiation. Interestingly, in an experiment comparing long day (LD) and short day (SD) conditions we found that “SA signalling module” gene expression was higher in SD grown plants. It was also seen that the SA gene induction upon cold treatment was time dependent, genes being more induced after longer times. An opposite pattern was observed in response to drought. Our comparison of abiotic stresses also showed that salinity has an inducible effect on the SA module in roots but not in shoots (Fig. 4).

10. Conclusion

SA is involved in and controls many physiological processes in plants, however it is best known as a master player in plant-microbe interactions. Recently, huge steps forward have been made in the understanding of SA signalling. Maybe one of the most important observations recently made concerns SA receptors. Because NPR1, NPR3, NPR4 proteins were described as SA receptors (Fu et al., 2012; Wu et al., 2012), Intriguing situation arises in connection with NPR1 which is both a SA receptor and a major SA main effector. But it is likely to be also true for other SA binding proteins, that have enzymatic activities, and which, therefore, are also SA effectors.

Concerning the control of SA level, data are quite abundant for the signalling pathway in response to biotic stresses. However, many questions remain unanswered. For instance, what is the in vivo activity of EDS1? Does EDS1 act on SA level and if so, by which mechanisms? Besides SA accumulation does not occur only after pathogen attack (biotic stress), but other abiotic stresses can contribute. Ozone, UV exposure, and H2O2 treatments increase SA accumulation (Leon et al., 1995; Neuschwander et al., 1995; Sharma et al., 1996; Surplus et al., 1998; Yalpani et al., 1994). The signalling pathways controlling SA levels in response to these abiotic conditions have been less studied compared to pathogen attack.

The studies of SA accumulating mutants illustrate that many processes can be involved in the regulation of SA level (Table 1). But to date, for most of these mutants, the way the altered proteins participate in SA control is not understood. An image is emerging where the SA level is constitutively negatively controlled by basal signalling pathways that give rise to a basal condition. Some of the bricks that make up these pathways might be genes affected in the SA overaccumulating mutants but how these bricks interact to form a given pathway is not known.

From study of SA overaccumulating mutants, a connection between sphingolipids and SA is made. The crosses with mutants impaired in SA biosynthesis and/or signalling can bring deeper insight in involvement of sphingolipids in the SA pathway. It would be of interest to know if the high SA in sphingolipids is EDS1-dependent.

A cornerstone of SA signal transduction is NPR1, the role of which is well established. NPR1-independent pathways also exist but data concerning the identification of components of these pathways are scarce. Therefore, this very exciting field of research could provide evidence showing the robustness of plant defence signalling. Finally, although SA plays a key role in SAR, the full understanding of how the signal spreads to other plant tissues and organs remains elusive.

A better knowledge of SA transduction pathways will also help build phytohormone regulatory networks that integrate the cross-talk between SA and jasmonic acid, ethylene, abscisic acid, auxin, cytokinines, gibberellic acid and brassinosteroids. This intriguing and complex phenomenon has not been highlighted since it has been reviewed elsewhere (Bari and Jones, 2009; Pieterse et al., 2009; Robert-Seilaniantz et al., 2011).

Other interesting areas are open to further investigation including the importance of SA in the regulation of plant growth and by which mechanisms SA can influence growth. Again, perhaps SA overaccumulating mutants will be good tools to answer such questions.

One of the main conclusions arising from the work on signalling events upstream and downstream from SA, is the fact that SA level can be controlled by phenomenon that also act downstream from SA. Redox status can alter SA level, while SA acts through the control of cell redox status. SA controls gene expression via lipid signalling, but lipid signalling also plays a role in controlling the level of SA. Indeed, when SA accumulating mutants are crossed with npr1 mutants, it can lead to mutants with an even higher SA level (Šálek et al., 2014). This shows that SA is under the control of a negative feedback loop driven by NPR1.

In conclusion, SA signalling is complex and over the coming years further advances will be required to better understand this fascinating magical mystery tour.

Acknowledgements

This research was supported by the Czech Science Foundation Grant No.501/11/1654 and Specific University Research (MSMT No. 21/2014) (M.J.). Martin Janda would like to thank Professor Olga Valentová and Associate Professor Lenka Burketová for their leadership and Dr. Kenichi Tsuda for his advice during his stay at the Max Planck Institute for Plant Breeding Research in Cologne. Eric Ruelland benefits from funding from the CNRS and the Université


Please cite this article in press as: Janda, M., Ruelland, E., Magical mystery tour: Salicylic acid signalling. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.07.003
Crane, O. Plemer, M. Vernglole, C. Collin, S. Renou, J.P. Taconnot, L. Yu, A. Bur- 
krin, E. Arnaud, C. Van- 
Kumar, D. Kessig, D.F. 2008. The search for the salicylic acid receptor led to 
Raju, S., Rivas, S. Roby, D. 2006. An essential role for salicylic acid in AMY3B0-
Mediated control of the hypersensitive cell death program in Arabidopsis. FEBS 
Letter. 580, 3498–3504.
Rainton, D., Humber, L. Delage, E. Vernglole, C. Cantrel, C. Mau/bert, M.A. Lan-
franco, S., Maldiney, R., Collin, S., Wolf, C., Zachowskii, A. Ruelland, E. 2012. Acyl 
chains of phosphatidase D transphosphatidylates products in arabidopsis cells: a 
study using multiple reaction monitoring mass spectrometry. PLOS ONE. 7, 
Ratcliffe, D. Delaney, T.F. 2002. Role of ARS signaling and NIM1/NPR1 in race-
Rao, M.V., Paliarth, C. Ormon, D.P. Marr, D.P. Watkins, C.B. Influence of sali-
Acid on H2O2 production, oxidative stress, and H2O2 metabolizing enzymes 
Rivas-San Vicente, M., Larios-Zarate, G., Rasenica, J. 2013. Disruption of sphin-
golipid biosynthesis in Niconia benthamiana activates salicylic acid-dependent 
responses and compromises resistance to Alternaria Alternari a f. sp. lycopersici. 
Robertson, A., Grant, M. Jones, J.D., 2011. Hormone crosstalk in plant dis-
ase and disease: more than just jasmonate–salicylate antagonism. Annu. Rev.
Saeki, V., Janda, M., Delage, E., Puyaubert, J. Guivarac, A. Lopez Maseda, E. Do-
brey, P.H. Caus, J. Roka, V. Puyaubert, J. Delage, E. Burketovskii, A. Ruelland, E. 
2014. Constitutive salicylic acid accumulation in pik4l1fl1f2 Arabidopsis plants 
Serrano, R., Lopez, B.J., Araya, M. Sang, J., Valen
tova, B., Nii and, S., Geisser, M., Mauch, F., Nawrath, C. Metraux, J.P. 2013. Export of salicylic acid from the chloroplast requires the multidrug and toxin 
extrusion-type transporter EDSS. Plant Physiol. 162, 1815–1821.
sensitive mutant (sal1) of Arabidopsis thaliana, identified in a selective screen 
utilizing the SA-inducible expression of the tms2 gene. Mol. Plant Microbe Interact. 
10 (1), 69–77.
sis SIZ2 gene confers SA- and NPR1-independent expression of PR genes and 
Shapiro, D., Zhang, C. 2001. The role of NPR1 in assurance gene-directed signaling 
Arabidopsis thaliana: the role of salicylic acid in the accumulation of defense-
Arabidopsis Toll Interleukin-1 Receptor-Nucleotide Binding Site-Leucine-Rich Re 
sponse motif (NBR1) gene triggers changes in gene expression and results in enhanced 
Simanshu, D.K., Zhai, X., Munch, D., Hofius, D., Markham, J.E., Bielawska, J., 
Bielawska, A., Malinina, M., Molotkovskiy, G.J., Mundy, J.W., et al. 2014. Arabidop-
sis acet 
ated cell death. An Arabidopsis receptor-like protein mediates cell death upon 
Song, F.M., Goodman, R.M. 2001. Activity of nitric oxide is dependent on, but is 
partially required for function of, salicylic acid in the signaling pathway in tobacco 
Spel, S.H., Loake, G.J. 2011. Redox-based protein modifications: the missing link in 
Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual 
roles in regulating plant cell death. Cell 137, 860–872.
Arabidopsis suppressor of cytokine signalling (SOCS) proteins and the regu-
lar 
salicylate biosynthesis exhibits properties consistent with a role in diverse stress 
Surprenant, C., Soble, B.R., Karrro, P., Thomas, B., Mackerness, S.A.H. 
acid and reactive oxygen species in the regulation of transcripts encoding phos-
thyosyntic and acidic pathogenesis-related proteins. Plant Cell Environ. 21, 
683–694.
Dong, X.N. 2008. Plant immunity requires coregulatory charges of NPR1 via 
protein docking site 321. 925–946.
development of several fungi on Arabidopsis can be reduced by treatment with 
technology identifies multiple salicylic acid-binding proteins. J. Plant 72, 1027–1038.
Please cite this article in press as: Janda, M., Ruelland, E., Magical mystery tour: Salicylic acid signalling. Environ. Exp. Bot. (2014), http://dx.doi.org/10.1016/j.envexpbot.2014.07.003

Further reading