Title

An update on salicylic acid biosynthesis, its induction and potential exploitation by plant viruses

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Abstract (111 words)

Salicylic acid (SA) is a plant hormone essential for effective resistance to viral and non-viral pathogens. SA biosynthesis increases rapidly in resistant hosts when a dominant host resistance gene product recognizes a pathogen. SA stimulates resistance to viral replication, intercellular spread and systemic movement. However, certain viruses stimulate SA biosynthesis in susceptible hosts. This paradoxical effect limits virus titer and prevents excessive host damage, suggesting that these viruses exploit SA-induced resistance to optimize their accumulation. Recent work showed that SA production in plants does not simply recapitulate bacterial SA biosynthetic mechanisms, and that the relative contributions of the shikimate and phenylpropanoid pathways to the SA pool differ markedly between plant species.

Article Highlights

- Salicylic acid (SA) stimulates plants to resist viral replication, cell-to-cell movement and systemic movement
- Recent work indicates that SA also contributes to meristem exclusion of viruses and symptom amelioration
- Certain viruses induce SA biosynthesis as they spread through susceptible hosts, suggesting they exploit SA-induced resistance to prevent over-accumulation and to moderate host damage
- Plant SA biosynthesis from isochorismate is completed in the cytosol, not in the plastid, and the relative importance of the shikimate versus phenylpropanoid pathways in SA biosynthesis varies between plants

Introduction: Salicylic acid has a central but ambiguous role in defense against viruses and other pathogens

In a groundbreaking paper, White [1**] showed that applying aspirin (acetylsalicylic acid), benzoic acid (BA) or salicylic acid (SA) solutions enhanced virus resistance and induced pathogenesis-related (PR) protein accumulation in plants of three tobacco mosaic virus (TMV)-resistant tobacco cultivars. PR proteins are known to effect resistance against certain cellular phytopathogens but at that time were suspected to be antiviral [2]. White's discoveries led to the realization that SA is a phytohormone required for induction of systemic acquired resistance (SAR: a pathogen-induced or stress-induced plant-wide enhancement of resistance to secondary infection by a variety of phytopathogens), for localization of pathogens to the infection site during hypersensitive responses (HRs) induced by resistance (*R*) gene-mediated effector-triggered immunity, and for maintenance of basal resistance [3,4,5,6].

Initial studies suggested that pathogen-induced SA biosynthesis was associated with necrosis occurring during the HR or caused by infection with necrotrophic pathogens such as *Colletotrichum lagenarium* [7,8]. However, subsequent work showed that certain viruses that spread systemically in hosts without causing necrosis can also induce SA accumulation [9,10,11,12]. Viruses that induce SA biosynthesis express factors that subvert SA-induced virus resistance, which explains how they can still replicate and spread. However, this provides no clarity as to whether SA accumulation is an incidental effect of infection, if it is somehow advantageous to the virus, or if it represents a delayed or ineffective resistance response. In this article, we review recent advances in the understanding of plant SA biosynthesis and how some viruses may exploit its induction to optimize their accumulation.

Plant salicylic acid biosynthetic pathways are distinct from those in bacteria

Soon after SA was shown to be an endogenous defensive signal, rapid progress was made in tracing its biosynthesis from intermediates in the phenylpropanoid pathway (Figure 1). In early work with tobacco and it was found that effective HR-type resistance to TMV, which is dependent upon SA, is inhibited in transgenic plants with decreased expression of phenylalanine ammonia-lyase (PAL), which catalyzes the initial step of the phenylpropanoid pathway [13]. SA can be synthesized by hydroxylation of the phenylpropanoid pathway product BA by a cytochrome P450 oxygenase, BA 2-hydroxylase [14,15] or, as later work suggested, from *ortho*-coumarate [16]. During this early research it was also found that SA is metabolized to methyl-SA, a volatile resistance inducer, and to biologically inactive forms (SA-β-D-glucoside or to a lesser extent to SA-glucose ester) that serve as vacuole-localized SA reserves [17,18] (Figure 1). Recent work indicated that the glycosylation status of di-hydroxylated SA metabolites helps regulate HR-related cell death [19**,20].

In 2001 SA biosynthesis research re-focused almost exclusively to the shikimate pathway as a source of SA precursors. This was stimulated by Wildermuth and colleagues' [21] discovery that plants of the SA-deficient Arabidopsis mutant line *SA induction-deficient 2* (*sid2*) were depleted in isochorismate synthase (ICS) activity. ICS catalyzes conversion of the shikimate pathway product chorismate to isochorismate (Figure 1). Arabidopsis chloroplasts contain two enzymatically active ICS isozymes with similar catalytic properties: ICS1, encoded by the wild-type *SID2* gene, and ICS2 [21,22]. ICS1 is translated from an inducible mRNA, transcription of which is stimulated by pathogen attack and auto-regulated by SA, whereas ICS2 is

produced constitutively at low levels [22,23]. ICS1 but not ICS2 is indispensable for effective pathogen resistance in Arabidopsis [21].

Bacteria use ICS in the first step of conversion of chorismate to SA, which they use in synthesis of iron-scavenging molecules called siderophores [24,25]. The second step of bacterial SA synthesis is conversion of isochorismate to SA, catalyzed by isochorismate pyruvate-lyase (IPL). Certain bacteria, including *Yersinia enterocolitica*, produce SA synthases: bifunctional proteins with ICS and IPL activities. Others (e.g. *Pseudomonas aeruginosa*) produce separate ICS and IPL enzyme molecules [24,25] (Figure 1). Several groups showed that plant ICS enzymes lack IPL activity (and are therefore not SA synthases) but attempts to identify *IPL*-like sequences in plant genomes proved unsuccessful [22]. A putative Arabidopsis *IPL* gene, encoding a protein with a sequence characteristic of a peroxidase (PRXR1), was detected by screening an Arabidopsis cDNA library using SA-responsive bacterial biosensors [26]. However, no work has been reported on SA biosynthesis in *prxr1* mutants, or if PRXR1 converts isochorismate to SA *in vitro*. Thus, PRXR1's conjectured IPL activity remains unconfirmed.

A recent exciting paper by Rekhter and colleagues [27**] indicates that in Arabidopsis complete synthesis of SA from chorismate does not require an IPL. Previous work had established that the protein ENHANCED DISEASE SUSCEPTIBILITY 5 (EDS5) transports SA across chloroplast envelopes [28]. The new paper reported that EDS5 also extrudes isochorismate from the chloroplast into the cytoplasm where it encounters an amidotransferase, avrPphB SUSCEPTIBLE 3 (PBS3) [27**]. PBS3 belongs to the Gretchen Hagen 3 group of proteins that catalyze formation of several phytohormone-amino acid conjugates. PBS3 is required for normal levels of SA accumulation [29,30,31] and can bind isochorismate

or chorismate [27**,32]. Rekhter et al. [27**] demonstrated that PBS3 catalyzes a condensation reaction between isochorismate and glutamate to produce isochorismate-9-glutamate, a conjugate that decomposes to SA and 2-hydroxy-acryloyl-N-glutamate (Figure 1). Thus, it now appears that plant biosynthesis of SA from chorismate is completed in the cytosol and is distinct from the bacterial IPL-dependent mechanism

At this time it appears that plants synthesize SA using carbon skeletons abstracted either from the shikimate or phenylpropanoid pathways. However, the proportion of total SA derived from each pathway differs between plant species. For instance, in Arabidopsis most SA is produced from chorismate via isochorismate and isochorismate-9-glutamate, with an additional <10% arising from the phenylpropanoid pathway [27**,33]. But in some dicots, such as tobacco and *Prunus*, SA arises predominantly from phenylpropanoid pathway activity [13,14,15,34]. Similar variation occurs in the grasses. For example, most SA in barley is synthesized from chorismate [35] whereas SA biosynthesis in maize is largely dependent upon PAL activity [36**]. Soybean is a particularly interesting case in that the shikimate and phenylpropanoid pathways are equally important in providing the carbon skeletons needed to generate sufficient SA to support defense against pathogens [37**].

Variation between plants that are mostly dependent upon ICS activity versus those dependent upon PAL activity for SA production may reflect specific metabolic needs or limitations in each plant, or the nature of external challenges (including viruses and other pathogens), or the degree of metabolic flexibility required to rise to various challenges. Chorismate is essential for production of several vital metabolites synthesized in chloroplasts, including aromatic amino acids, folate and

phylloquinone [18]. Some plants may not have sufficient metabolic flexibility to be able to maintain synthesis of these compounds while drawing on what might be a limited chorismate pool to synthesize SA.

Salicylic acid-induced resistance to viruses: Still not fully understood

We recently reviewed the topic of SA-induced resistance to viruses and how it connects with resistance mechanisms regulated by signals such as jasmonic acid, abscisic acid, azelaic acid, glycerol-3-phosphate, nitric oxide, reactive oxygen species (ROS) and pipecolic acid [2]. Therefore, the mechanisms suspected to be involved in SA-induced resistance will only be summarized here (Figure 2).

For the most part SA influences virus resistance by acting as a signal over various ranges to stimulate genetic and physiological changes in the plant. An exception to this occurs in the case of the viral replicase complex of tomato bushy stunt virus, where SA binds directly to a host factor, a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) isoform, required for regulating the ratio of viral genomic (plus-sense) to viral minus-strand synthesis [38*]. In SA-treated tobacco the relative proportions of minus and plus strands of TMV RNA and of sub-genomic mRNAs synthesized were also altered. But in that plant-virus combination the effect of SA on viral RNA synthesis was indirect and mediated by defensive signaling modulated by the mitochondrial respiratory enzyme, alternative oxidase (AOX) [39] (Figure 2).

AOX and AOX-like enzymes occur in mitochondria of plants, certain fungi, invertebrates and proteobacteria, but not in mitochondria of higher vertebrates [40*]. Plant AOX is an accessory respiratory chain component that prevents over-reduction of ubiquinone, neutralizes excess reducing power from photosynthesis, and moderates mitochondrial ROS accumulation [40*]. AOX uses ubiquinol to catalyze

reduction of oxygen to water, without concomitant generation of ATP [40*]. There are multiple examples of virus-plant interactions in which AOX is a factor in SA-induced virus resistance (reviewed in [2]).

Modulation of mitochondrial ROS by AOX is theorized to affect nuclear gene expression via retrograde signaling. This probably involves signaling transduced via reversible oxidation of sulfhydryl groups and reduction of disulfide bridges on mitochondrial sensor proteins [41]. SA stimulates mitochondrial ROS production by interactions with α-ketoglutarate dehydrogenase and/or inhibition of electron transport [2,42*]. Increased mitochondrial ROS levels activate AOX activity and a transient increase in AOX gene expression to counteract further ROS production [2,42*]. Consistent with this idea, altering glutathione levels can compensate for decreased SA accumulation in induction of virus resistance [43]. However, AOX is not always a factor in SA-induced virus resistance. While SA-induced resistance is modulated by AOX in Arabidopsis, tobacco and *N. benthamiana*, it is AOX-independent in squash [2,44,45] (Figure 2).

SA-induced virus resistance is not dependent on any known PR protein and in most cases is not dependent on NPR1 (‘Non-Expresser of PR proteins 1’), a regulator of *PR* gene expression (reviewed in [2]) (Figure 2). However, NPR1 is implicated in two examples of virus resistance. One is virus localization during the HR [46]. The second is the suggested role of NPR1 in resistance induced by the SA analog benzothiadiazole against plantago asiatica mosaic virus in Arabidopsis [47]. SA can induce resistance to viral replication, cell-to-cell movement, and systemic movement. But which step of the infection cycle is inhibited depends upon the virus-host combination [2,6,45,47].

SA treatment can limit access of viruses to tissues adjacent to the meristem; the growing tip where most cell division and differentiation occurs [48]. The extent of viral invasion of meristematic tissue correlates with symptom severity [48,49]. Until recently, meristem access was thought to be controlled predominantly by RNA silencing mediated by RNA-dependent RNA polymerase (RDR) 6 (which is not SA-regulated) and RDR1, which SA induces at the transcriptional level and activates at the enzymatic level [48,49]. Although RNA silencing and its reinforcement by SA explains exclusion of TMV and potato virus X from meristems and symptom amelioration [48,49], Medzihradszky and colleagues [50**] contend that for tombusviruses, such as cymbidium ringspot virus, virus-induced changes in host gene expression are more important for exclusion. Most significantly, they point to decreased gene expression for GAPDH, which, as previously noted, is not only a host factor required for efficient tombusvirus replication but is also a target for SA [38*,50**] (Figure 2).

RDR1 is an ancillary RNA silencing component that maintains basal resistance against several viruses and SA enhances its expression in an NPR1-dependent fashion [23,48,51]. However, neither RDR1, nor core RNA silencing components such as the endonucleases Dicer-like (DCL) 2, 3, or 4 are essential for resistance induced by SA or its functional analogs [47,52]. Thus, SA-induced virus resistance is not dependent upon RNA silencing. However, RDR1 enhances expression of RDR6, AOX and of a suspected antiviral factor (Inhibitor of Viral Replication [51]). Taken together with data showing that *RDR1* expression, but not *AOX* expression, is regulated by NPR1 [23], it seems that a complex but incompletely elucidated regulatory network coordinates SA-induced resistance with other aspects of SAR and with RNA silencing (Figure 2).

Balancing act: Salicylic acid as a pro-viral factor

Treatment of susceptible plants with exogenous SA, synthetic resistance inducers or induction of endogenous SA biosynthesis prior to inoculation inhibits infection by most viruses, although it is not as effective as ETI in completely preventing infection [2,6]. Paradoxically, some of the viruses that would be inhibited in some aspect of their infection cycle in plants pre-treated with SA can induce SA biosynthesis, although this does not prevent infection (Figure 3). Examples include potyviruses, cucumber mosaic virus (CMV) and cauliflower mosaic virus (CaMV), which induce SA biosynthesis during compatible interactions with plants [9,10,11,36**,53].

Probably the best-studied viral factors that enable viruses to overcome at least some aspects of SA-induced resistance include the CMV 2b protein [54,55], the potyviral HC-Pro protein [56,57*] and the P6 protein of CaMV [11]. Interestingly, these viral gene products also enable their respective viruses to overcome RNA silencing, and provoke disease symptoms through interference with small RNA pathways as well as via other mechanisms [58,59]. Two amino acid sequences within P6 condition suppression of SA-mediated signaling by CaMV [60]. For the 2b protein, the N- and C-terminal domains are required for evasion of SA-induced resistance to local virus accumulation. These domains, plus the region containing superimposed nuclear localization and RNA binding sequences, and the central gly-ser-glu-leu sequence contribute to priming of SA biosynthesis, which is induced by another, unidentified CMV gene product. The phosphorylation (nucleus-cytoplasm shuttling) domain negatively regulates SA biosynthesis [10,55,61]. For potyviruses, HC-Pro both induces SA biosynthesis and allows potyviruses to evade the antiviral effects of SA, with inhibition of downstream signaling caused by interaction with SA-binding protein 3 [57*,62].

Recently, it was found that the tobacco rattle virus (TRV) 16K protein induces SA biosynthesis and expression of *RDR1* and other SA-regulated genes in systemically infected *N. benthamiana* plants [63**]. Mechanistically, the process hinges on interaction of the 16K protein with the host protein coilin, leading to coilin's relocation from the intra-nuclear Cajal bodies to the nucleoli, which triggers SA-induced resistance to further TRV accumulation [63**]. Once invoked, this process prevents significant accumulation of TRV in young, developing tissues, which display no discernable symptoms: a recovery phenotype. When TRV-induced SA accumulation was hindered by transgenic expression of the SA-degrading enzyme SA hydroxylase, knockdown of *coilin* expression, or infection with a TRV *16K*-deletion mutant, infected plants exhibited aggravated symptoms culminating in necrosis [63**].

Shaw and colleagues [63**] showed that recovery, previously attributed solely to RNA silencing (critically reviewed in [64]), is SA-dependent and that, rather than being a pure resistance phenomenon, may represent viral manipulation of host resistance to optimize virus accumulation, whilst limiting damage to the host. Other evidence for viral self-limitation and symptom amelioration by inducing SA biosynthesis is provided by studies where transgenic expression of SA hydroxylase led to increased pathogenicity in potato virus Y-infected potato plants [53], and in *PAL*-depleted maize plants infected with sugarcane mosaic virus [36**]. SA might be considered to be pro-viral where it facilitates limitation of virus accumulation to avoid excessive host damage such as necrosis, which would inactivate virus particles in dying tissues or might render hosts unattractive to vectors (Figure 3).

Concluding comments: Future studies of SA-induced resistance

Although SA-induced virus resistance occurs independently of RNA silencing, it appears that these two phenomena reinforce each other [48,56] and are linked, perhaps through the action of RDR1 [51]. It is plausible that SA accumulation in virus infected plants primes RNA silencing. This is suggested by observations that in transgenic Arabidopsis plants expressing the CMV 2b protein *AGO2* expression becomes SA-inducible [10], and that AGO2 provides a second line of defense against CMV [65]. Priming of RNA silencing by SA, whether though induction and activation of RDR1, or by increasing core components of silencing such as AGO2 would strengthen SA-induced resistance (Figure 3a) but may also be exploitable by viruses to control their own accumulation (Figure 3b). Further research on the SA - RNA silencing linkage is likely to yield important new insights into plant-virus relationships.

Work on the tobacco-TMV pathosystem suggested that in general SA accumulation is not induced during infection of susceptible plants [7]. However, virus-induced SA accumulation has now been observed in many susceptible hosts, which suggest that this may be the rule, and that the TMV-tobacco system might be an exception. Further research in this area may reveal additional functions for virus-induced SA accumulation in infected plants beyond modulation of virus titer. Aguilar and colleagues have shown that SA is needed to establish virus-induced drought resistance [66] and that virus-induced SA accumulation protects plants against secondary infection by bacteria [67**]. Both effects have mutual benefits for host and virus and it is conceivable that SA will prove to be a key factor in facilitating quasi-mutualistic ‘pay-backs’ between viruses and their hosts.

Conflicts of interest

All authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Author declaration

All authors reviewed the final draft. The corresponding author had final responsibility for the decision to submit the manuscript for publication.

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References and Recommended Reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. ** White RF: **Acetylsalicylic acid (Aspirin) induces resistance to tobacco mosaic virus in tobacco.** *Virology* 1979, **99**:410-412. DOI: 10.1016/0042-6822(79)90019-9

Although published forty years ago, this paper is still worth reading and marks the beginning of modern studies of defensive signaling in plants and shows that salicylic as well as its synthetic derivative aspirin are powerful resistance inducers.

2. Carr JP, Murphy AM, Tungadi T, Yoon JY: **Plant defense signals: Players and pawns in plant-virus-vector interactions.** *Plant Science* 2019, **279**:87-95. DOI:10.1016/j.plantsci.2018.04.011

3. Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, Ryals J: **A central role of salicylic acid in plant disease resistance**. *Science* 1994, **266**:1247–1250. DOI:10.1126/science.266.5188.1247

4. Moffett P: **Transfer and modification of NLR proteins for virus resistance in plants.** *Current Opinion in Virology* 2017, **26:**43-48. DOI: 10.1016/j.coviro.2017.07.008

5. Mur LAJ, Bi Y, Darby RM, Firek S, Draper J: **Compromising early salicylic acid accumulation delays the hypersensitive response and increases viral dispersal during lesion establishment in tobacco mosaic virus–infected tobacco.** *Plant Journal* 1997, **12**:1113–1126. DOI:10.1046/j.1365-313X.1997.12051113.x

6. Palukaitis P, Yoon JY, Choi SK, Carr JP: **Manipulation of induced resistance to viruses.** *Current Opinion in Virology* 2017, **26:**141–148. doi: 10.1016/j.coviro.2017.08.001

7. Malamy J, Carr JP, Klessig DF, Raskin I: **Salicylic acid - A likely endogenous signal in the resistance response of tobacco to viral infection.** *Science* 1990, **250**:1002-1004. DOI: 10.1126/science.250.4983.1002

8. Métraux JP, Signer H, Ryals J, Ward E, Wyss-Benz M, Gaudin J, Raschdorf K, Schmid E, Blum W, Inverardi B: **Increase in salicylic acid at the onset of systemic acquired resistance in cucumber.** *Science* 1990, **250**:1004-1006. DOI: 10.1126/science.250.4983.1004

9. Krečič-Stres H, Vucak C, Ravnikar M, Kovač M: **Systemic *Potato virus Y*^{NTN} infection and levels of salicylic and gentisic acids in different potato**

genotypes. *Plant Pathology* 2005, **54**:441-447. DOI:10.1111/j.1365-3059.2005.01209.x

10. Lewsey MG, Murphy AM, MacLean D, Dalchau N, Westwood JH, Macaulay K, Bennett MH, Moulin M, Hanke DE, Powell G, Smith AG, Carr JP: **Disruption of two defensive signaling pathways by a viral RNA silencing suppressor.** *Molecular Plant-Microbe Interactions* 2010, **23**:835-845. DOI: 10.1094/MPMI-23-7-0835

11. Love AJ, Geri C, Laird J, Carr C, Yun B-W, Loake GJ, Tada Y, Sadanandom A, Milner JJ: ***Cauliflower mosaic virus* protein P6 inhibits signaling responses to salicylic acid and regulates innate immunity.** *PLoS ONE* 2012, **7(10)**:e47535. DOI: 10.1371/journal.pone.0047535

12. Xu P, Chen F, Mannas JP, Feldman T, Sumner LW, Roossinck MJ: **Virus infection improves drought tolerance.** *New Phytologist* 2008, **180**:911–921. DOI: 10.1111/j.1469-8137.2008.02627.x

13. Pallas JA, Paiva NL, Lamb C, Dixon RA: **Tobacco plants epigenetically suppressed in phenylalanine ammonia lyase expression do not develop systemic acquired resistance in response to infection by tobacco mosaic virus.** *Plant Journal* 1996, **10**: 281-293. doi.org/10.1046/j.1365-313X.1996.10020281.x

14. León J, Shulaev V, Lawton MA, Raskin I: **Benzoic acid 2-hydroxylase, a soluble oxygenase from tobacco, catalyzes salicylic acid biosynthesis.** *Proc Natl Acad Sci USA* 1995, **92**:10413–10417. doi: 10.1073/pnas.92.22.10413

15. Yalpani N, Leon J, Lawton MA, Raskin I: **Pathway of salicylic acid biosynthesis in healthy and virus-inoculated tobacco.** *Plant Physiology* 1993, **103**:315-321. DOI: 10.1104/pp.103.2.315

16. Malinowski J, Krzymowska M, Godon K, Hennig J, Podstolski A: **A new catalytic activity from tobacco converting 2-coumaric acid to salicylic aldehyde.** *Physiologia Plantarum* 2007, **129**:461–471. DOI: 10.1111/j.1399-3054.2006.00837.x

17. Lee HI, Leon J, Raskin I: **Biosynthesis and metabolism of salicylic acid**. *Proc Natl Acad Sci USA* 1995, **92**:4076–4079. DOI:10.1073/pnas.92.10.4076

18. Widhalm JR, Dudareva N: **A familiar ring to it: Biosynthesis of plant benzoic acids.** *Molecular Plant* 2015, **8**:83–97 DOI: 10.1016/j.molp.2014.12.001

19. ****** Huang X, Zhu G, Liu Q, Chen L, Li Y, Hou B: **Modulation of plant salicylic acid-associated immune responses via glycosylation of dihydroxybenzoic acids**. *Plant Physiology* 2018, 176:3103-3119. DOI:10.1104/pp.17.01530

The work in this paper indicates that glucosylated and hydroxylated derivatives of salicylic acid are neither merely SA metabolites nor storage compounds but play a dynamic role in resistance and cell death induction in the hypersensitive response.

20. Zhang Y, Zhao L, Zhao J, Li Y, Wang J, Guo R, Gan S, Liu C, Zhang K: **S5H/DMR6 encodes a salicylic acid 5-hydroxylase that fine-tunes salicylic acid homeostasis.** *Plant Physiology* 2017, **175:**1082–1093. DOI: 10.1104/pp.17.00695

21. Wildermuth MC, Dewdney J, Wu G, Ausubel FM: **Isochorismate synthase is required to synthesize salicylic acid for plant defence.** *Nature* 2001, **414**:562–565 doi:10.1038/35107108

22. Macaulay KM, Heath GA, Ciulli A, Murphy AM, Abell C, Carr JP, Smith AG: **The biochemical properties of the two *Arabidopsis thaliana* isochorismate synthases**. *Biochemical Journal* 2017, **474**:1579-1590. DOI: 10.1042/BCJ20161069

23. Hunter LJR, Westwood JH, Heath G, Macaulay K, Smith AG, MacFarlane SA, Palukaitis P, Carr JP: **Regulation of *RNA-dependent RNA polymerase 1* and *Isochorismate Synthase* gene expression in Arabidopsis.** *PLoS ONE* 2013, **8(6)**: e66530. DOI:10.1371/journal.pone.0066530

24. Kerbarh O, Ciulli A, Howard NI, Abell C: **Salicylate biosynthesis: overexpression, purification and characterization of Irp9, a bifunctional salicylate synthase from *Yersinia enterocolitica*.** *Journal of Bacteriology* 2005, **187**:5061–5066 DOI:10.1128/JB.187.15.5061-5066.2005

25. Serino L, Reimmann C, Baur H, Beyeler M, Visca P, Haas D: **Structural genes for salicylate biosynthesis from chorismate in *Pseudomonas aeruginosa*.** Mol Gen Genet 1995, **249**:217–228 doi:10.1007/BF00290369

26. Zhou Y, Memelink J, Linthorst HJM: **An *E. coli* biosensor for screening of cDNA libraries for isochorismate pyruvate lyase-encoding cDNAs.** *Molecular Genetics and Genomics* 2018, **293:**1181–1190. DOI: 10.1007/s00438-018-1450-5

27. ****** Rekhter D, Lüdke D, Ding Y, Feussner K, Zienkiewicz K, Lipka V, Wiermer M, Zhang Y, Feussner I: **Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid.** *Science* 2019, **365**:498–502. Doi: 10.1126/science.aaw1720

This important paper overthrows the paradigm that the plant biosynthetic route from chorismate to salicylic acid recapitulates bacterial salicylic acid biosynthesis and reveals a novel mechanism for completion of biosynthesis occurring in the cytosol that is independent of isochorismate pyruvate-lyase activity.

28. Serrano M, Wang B, Aryal B, Garcion C, Abou-Mansour E, Heck S, Geisler M, Mauch F, Nawrath C, Métraux JP: **Export of salicylic acid from the chloroplast requires the multidrug and toxin extrusion-like transporter EDS5**. *Plant Physiology* 2013, **165**:1815-1821 doi:10.1104/pp.113.218156.

29. Jagadeeswaran G, Raina S, Acharya BR, Maqbool SB, Mosher SL, Appel HM, Schultz JC, Klessig DF, Raina R: **Arabidopsis *GH3-like defense gene 1* is required for accumulation of salicylic acid, activation of defense responses and resistance to *Pseudomonas syringae***. *Plant Journal* 2007, **51**:234–246. DOI: 10.1111/j.1365-313X.2007.03130.x

30. Nobuta K, Okrent RA, Stoutemyer M, Rodibaugh N, Kempema L, Wildermuth MC, Innes RW: **The GH3 acyl adenylase family member PBS3 regulates salicylic acid-dependent defense responses in *Arabidopsis*.** *Plant Physiology* 2007, **144**:1144–1156. DOI:10.1104/pp.107.097691

31. Okrent RA, Brooks MD, Wildermuth MC: **Arabidopsis GH3.12 (PBS3) conjugates amino acids to 4-substituted benzoates and is inhibited by salicylate.** *Journal of Biological Chemistry* 2009, **284**:9742–9754. DOI: 10.1074/jbc.M806662200

32. Holland CK, Westfall CS, Schaffer JE, De Santiago A, Zubieta C, Alvarez S, Jez JM: ***Brassicaceae*-specific Gretchen Hagen 3 acyl acid amido synthetases conjugate amino acids to chorismate, a precursor of aromatic amino acids and salicylic acid.** *Journal of Biological Chemistry* 2019, **294(45)**: 16855–16864. DOI:10.1074/jbc.RA119.009949

33. Huang J, Gu M, Lai Z, Fan B, Shi K, Zhou Y, Yu J, Chen Z: **Functional analysis of the *Arabidopsis PAL* gene family in plant growth, development, and response to environmental stress.** *Plant Physiology* 2010, **153**:1526–1538. DOI: 10.1104/pp.110.157370.

34. Diaz-Vivancos, P., Bernal-Vicente, A., Cantabella, D., Petri, C. and Hernandez JA: **Metabolomics and biochemical approaches link salicylic acid biosynthesis to cyanogenesis in peach plants.** *Plant Cell Physiology* 2017, **58**:2057–2066. DOI: 10.1093/pcp/pcx135

35. Hao QQ, Wang WQ Han XL, Wu JZ, Lyu B, Chen FJ, Caplan A, Li CX, Wu JJ, Wang W, Xu Q, Fu DL: **Isochorismate-based salicylic acid biosynthesis confers basal resistance to *Fusarium graminearum* in barley.** *Molecular Plant Pathology* 2018, **19:**1995-2010. DOI: 10.1111/mpp.12675

36. ** Yuan W, Jiang T, Du K, Chen H, Cao Y, Xie J, Li M, Carr JP, Wu B, Fan Z, Zhou T: **Maize phenylalanine ammonia-lyases contribute to resistance to *Sugarcane mosaic virus* infection, most likely through positive regulation of salicylic acid accumulation.** *Molecular Plant Pathology* 2019 DOI: 10.1111/mpp.12817

The paper shows that in the important crop maize phenylalanine ammonia-lyase activity is essential for salicylic acid synthesis, rather than isochorismate synthase as in barley [35], showing there is variation between grasses. The paper also shows that a virus can manipulate phenylalanine ammonia-lyase activity and salicylic acid biosynthesis to moderate its titer.

37. ** Shine MB, Yang JW, El-Habbak M, Nagyabhuru P, Fu DQ, Navarre D, Ghabrial S, Kachroo P, Kachroo A: **Cooperative functioning between phenylalanine ammonia lyase and isochorismate synthase activities contributes to salicylic acid biosynthesis in soybean.** *New Phytologist* 2016, **212**:627–636 doi:10.1111/nph.14078

An important paper that shows how one plant, soybean, can utilize both plant biosynthetic routes for salicylic acid; dependent on phenylalanine ammonia-lyase versus isochorismate synthase.

38. * Tian M, Sasvari Z, Gonzalez PA, Friso G, Rowland E, Liu XM, van Wijk KJ, Nagy PD, Klessig DF: **Salicylic acid inhibits the replication of tomato**

bushy stunt virus by directly targeting a host component in the replication complex. *Molecular Plant-Microbe Interactions* 2015, **28**:379-386. DOI: 10.1094/MPMI-09-14-0259-R

This is the only documented example where salicylic acid appears to have a direct influence on the replication of a virus. In this case through its interaction with a host factor in the tombusvirus replication complex.

39. Chivasa S, Murphy AM, Naylor M, Carr JP: **Salicylic acid interferes with tobacco mosaic virus replication *via* a novel, salicylhydroxamic acid-sensitive mechanism.** *Plant Cell* 1997, **9**:547-557. DOI: 10.1105/tpc.9.4.547

40. ***** Rogov AG, Sukhanova EI, Uralskaya LA, Aliverdieva DA, Zvyagilskaya RA: **Alternative oxidase: Distribution, induction, properties, structure, regulation, and functions**. *Biochemistry* (Moscow) 2014, **79**: 1615-1634. DOI: 10.1134/S0006297914130112

A very fine review article on the mode of action of alternative oxidases and their occurrence in plants, fungi and lower animals.

41. Singh DP, Moore CA, Gilliland A, Carr JP: **Activation of multiple anti-viral defence mechanisms by salicylic acid.** *Molecular Plant Pathology* 2004, **5**:57-63. DOI: 10.1111/j.1364-3703.2004.00203.x

42. ***** Klessig DF, Tian M, Choi HW: **Multiple targets of salicylic acid and its derivatives in plants and animals.** *Frontiers in Immunology*, 2016 **7**:206. DOI: 10.3389/fimmu.2016.00206

An excellent review article on salicylic acid, its potential modes of action in plants and a discussion of its still incompletely understood pharmacological effects in animals.

43. Künstler A, Király L, Kátay G, Enyedi AJ, Gullner G: **Glutathione can compensate for salicylic acid deficiency in tobacco to maintain resistance to tobacco mosaic virus.** *Frontiers in Plant Science* 2019, **10**:1115. DOI: 10.3389/fpls.2019.01115

44. Lee WS, Fu SF, Verchot-Lubicz J, Carr JP: **Genetic modification of alternative respiration in *Nicotiana benthamiana* affects basal and salicylic acid-induced resistance to potato virus X.** *BMC Plant Biol* 2011, **11**:41. DOI: 10.1186/1471-2229-11-41

45. Mayers CN, Lee KC, Moore CA, Wong SK, Carr JP: **Salicylic acid-induced resistance to *Cucumber mosaic virus* in squash and *Arabidopsis thaliana*: Contrasting mechanisms of induction and antiviral action.** *Molecular Plant-Microbe Interactions* 2005, 18:428-434. DOI:10.1094/MPMI-18-0428

46. Liu YL, Schiff M, Marathe R, Dinesh-Kumar SP: **Tobacco *Rar1*, *EDS1* and *NPR1/NIM1* like genes are required for N-mediated resistance to tobacco mosaic virus**. *Plant Journal* 2002, **30**:415-429. DOI: 10.1046/j.1365-313X.2002.01297.x

47. Matsuo Y, Novianti F, Takehara M, Fukuhara T, Arie T, Komatsu K: **Acibenzolar-S-methyl restricts infection of *Nicotiana benthamiana* by**

plantago asiatica mosaic virus at two distinct stages. *Molecular Plant-Microbe Interactions* 2019, **32**:1475-1486. DOI: 10.1094/MPMI-03-19-0087-R

48. Lee WS, Fu SF, Li Z, Murphy AM, Dobson EA, Garland L, Chaluvadi SR, Lewsey MG, Nelson RS, Carr JP: **Salicylic acid treatment and expression of an RNA-dependent RNA polymerase 1 transgene inhibits lethal symptoms and meristem invasion during tobacco mosaic virus infection in *Nicotiana benthamiana*.** *BMC Plant Biol* 2016, **16**:15 DOI: 10.1186/s12870-016-0705-8.

49. Schwach F, Vaistij FE, Jones L, Baulcombe DC: **An RNA-dependent RNA polymerase prevents meristem invasion by potato virus X and is required for the activity but not the production of a systemic silencing signal.** *Plant Physiol* 2005, **138**:1842–52. doi: 10.1104/pp.105.063537

50. ** Medzihradszky A, Gyula P, Sós-Hegedűs A, Szittya G, Burgyán J: **Transcriptome reprogramming in the shoot apical meristem of CymRSV-infected *Nicotiana benthamiana* plants associates with viral exclusion and the lack of recovery.** *Molecular Plant Pathology* 2019, DOI:10.1111/mpp.12875

This study describes another mechanism (in addition to RNA silencing) for prevention of viral invasion of the plant meristematic tissue and symptom amelioration. For tombusviruses this may involve the same antiviral mechanism elucidated in reference [38*].

51. Rakhshandehroo F, Takeshita M, Squires J, Palukaitis P: **The influence of RNA-dependent RNA polymerase 1 on *Potato virus Y* infection and on other antiviral response genes**. Molecular Plant-Microbe Interactions 2009, 22:1312-1318. DOI:10.1094 /MPMI -22-10-1312.

52. Lewsey MG, Carr JP: **Effects of DICER-like proteins 2, 3 and 4 on cucumber mosaic virus and tobacco mosaic virus infections in salicylic acid-treated plants.** *Journal of General Virology* 2009, **90**:3010–3014. DOI 10.1099/vir.0.014555-0

53. Baebler Š, Stare K, Kovač M, Blejec A, Prezelj N, Stare T, Kogovšek P, Pompe-Novak M, Rosahl S, Ravnikar M, Gruden K: **Dynamics of responses in compatible potato - *Potato virus Y* interaction are modulated by salicylic acid.** *PLoS ONE* 2011, **6(12)**:e29009. doi:10.1371/journal.pone.0029009

54. Ji LH, Ding SW: **The suppressor of transgene RNA silencing encoded by *Cucumber mosaic virus* interferes with salicylic acid-mediated virus resistance.** *Molecular Plant-Microbe Interactions* 2001, **14**:715–724. DOI: 10.1094/MPMI.2001.14.6.715

55. Zhou T, Murphy AM, Lewsey MG, Westwood JH, Zhang H, González I, Canto T, Carr JP: **Domains of the cucumber mosaic virus 2b silencing suppressor protein affecting inhibition of salicylic acid-induced resistance and priming of salicylic acid accumulation during infection.** *Journal of General Virology* 2014, **95:**1408-1413. DOI: 10.1099/vir.0.063461-0

565 56. Alamillo JM, Saénz P, García JA: **Salicylic acid-mediated and RNA-**
566 **silencing defense mechanisms cooperate in the restriction of systemic**
567 **spread of plum pox virus in tobacco.** *Plant Journal* 2006, **48**:217–227.
568 DOI:10.1111/j.1365-313X.2006.02861.x

569 57. ***** Poque S, Wu HW, Huang CH, Cheng HW, Hu WC, Yang JY, Wang D, Yeh
570 SD: **Potyviral gene-silencing suppressor HCPro interacts with salicylic**
571 **acid (SA)-binding protein 3 to weaken SA-mediated defense responses.**
572 *Molecular Plant-Microbe Interactions* 2018, **31:**86-100. doi:10.1094/MPMI-06-
573 17-0128-FI

574 This paper provides a mechanistic explanation of how one of the most multi-functional of viral proteins,
575 the potyviral HC-Pro protein interferes with induction of pathogen resistance by salicylic acid.

576 58. Du Z, Chen A, Chen W, Liao Q, Zhang H, Bao Y, Roossinck MJ, Carr JP:
577 **Nuclear-cytoplasmic partitioning of the *Cucumber mosaic virus* 2b**
578 **protein determines the balance between its roles as a virulence**
579 **determinant and RNA silencing suppressor.** *Journal of Virology* 2014,
580 **88**:5228–5241. DOI: 10.1128/JVI.00284-14

581 59. Mlotshwa S, Pruss GJ, MacArthur JL, Reed JW, Vance V: **Developmental**
582 **defects mediated by the P1/HC-Pro potyviral silencing suppressor are**
583 **not due to misregulation of AUXIN RESPONSE FACTOR 8.** *Plant*
584 *Physiology* 2016, 172:1853–1861. DOI:10.1104/pp.16.01030

585 60. Laird J, McInally C, Carr C, Doddiah S, Yates G, Chrysanthou E, Khattab A,
586 Love AJ, Geri C, Sadanandom A, Smith BO, Kobayashi K, Milner JJ:
587 **Identification of the domains of cauliflower mosaic virus protein P6**
588 **responsible for suppression of RNA silencing and salicylic acid**
589 **signalling.** *Journal of General Virology* 2013, **94**:2777–2789. DOI:
590 10.1099/vir.0.057729-0

591 61. Carr JP, Murphy AM: **Suppression of Plant Defense.** In Cucumber Mosaic
592 Virus. 2019. Eds. Palukaitis P, García-Arenal F. APS Press St. Paul MN
593 **ISBN:** 978-0-89054-610-9, pp. 47–58. DOI:10.1094/9780890546109.007

594 62. Yang LP, Xu YN, Liu YQ, Meng DW, Jin TC, Zhou XF: **HC-Pro viral**
595 **suppressor from tobacco vein banding mosaic virus interferes with DNA**
596 **methylation and activates the salicylic acid pathway.** *Virology* 2016,
597 **497:**244-250. DOI: 10.1016/j.virol.2016.07.024

598 63. ****** Shaw J, Yu C, Makhotenko AV, Makarova SS, Love AJ, Kalinina NO,
599 MacFarlane S, Chen J, Taliansky ME: **Interaction of a plant virus protein**
600 **with the signature Cajal body protein coilin facilitates salicylic acid-**
601 **mediated plant defence responses.** *New Phytologist* 2019, **224**:439–453.
602 doi: 10.1111/nph.15994

603 This study reveals how interactions between the tobacco rattle virus 16K protein and a nuclear protein,
604 coilin, triggers salicylic acid biosynthesis and antiviral resistance and prevents excessive virus-
605 induced damage to the host plant.

606 64. Ghoshal B, Sanfaçon H: **Symptom recovery in virus-infected plants:**
607 **Revisiting the role of RNA silencing mechanisms.** *Virology* 2015, **479–**
608 **480**:167–179. DOI: 10.1016/j.virol.2015.01.008

609 65. Harvey JJW, Lewsey MG, Patel K, Westwood J, Heimstädt S, Carr JP,
610 Baulcombe DC: **An antiviral defence role of AGO2 in plants.** *PLoS One*
611 2011, **6(1)**: e14639. DOI: 10.1371/journal.pone.0014639

612 66. Aguilar E, Cutrona C, del Toro FJ, Vallarino JG, Osorio S, Pérez-Bueno ML,
613 Barón M, Chung BN, Canto T, Tenllado F: **Virulence determines beneficial**
614 **trade-offs in the response of virus-infected plants to drought via**
615 **induction of salicylic acid.** *Plant, Cell and Environment* 2017, **40**:2909–2930
616 DOI: 10.1111/pce.13028

617 67. ** Aguilar E, del Toro FJ, Figueira-Galán D, Hou W, Canto T, Tenllado F:
618 **Virus infection induces resistance to *Pseudomonas syringae* and to**
619 **drought in both compatible and incompatible bacteria–host interactions,**
620 **which are compromised under conditions of elevated temperature and**
621 **CO_2 levels.** *Journal of General Virology* 2020, **101**:122-135. DOI:
622 10.1099/jgv.0.001353

623 Following on from their study [66] showing that virally induced salicylic acid biosynthesis provides
624 protection against drought, the authors show that this also decreases susceptibility to secondary
625 infection by a bacterial pathogen. This may represent another manifestation of a virus-to-host
626 'payback'.

627 68. Torrens-Spence MP, Bobokalonova A, Carballo V, Glinkerman CM, Pluskal T,
628 Shen A, Weng JK: **PBS3 and EPS1 complete salicylic acid biosynthesis**
629 **from isochorismate in Arabidopsis.** *Molecular Plant* 2019, 12:1577–1586.
630 DOI: 10.1016/j.molp.2019.11.005

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Figure Legends

Figure 1. Biosynthetic Pathways for Production of Salicylic Acid in Plants and Bacteria. **(a)** In plants SA biosynthesis can utilize carbon skeletons derived from either or both of the shikimate or phenylpropanoid pathways. The relative importance of each of these pathways varies between plant species. SA derived from the phenylpropanoid pathway and dependent upon the conversion of phenylalanine to *trans*-cinnamic acid by PAL and subsequent conversion by either of two CoA-dependent routes or a CoA-independent route to BA, which is converted to SA by the action of a cytochrome P450 enzyme, BA2H, using molecular oxygen. SA produced from carbon skeletons provided by the shikimate pathway is derived from isochorismate produced in the plastid. Isochorismate is translocated into the cytosol by EDS5 and conjugated to glutamate by PBS3. The resulting compound, isochorismate-9-glutamate, decomposes to release SA and 2-hydroxy-acryloyl-N-glutamate (1). Alternatively, but only in Brassicaceae, EPS can catalyze decomposition of isochorismate-9-glutamate to N-pyruvoyl-L-glutamate (an 2-hydroxy-acryloyl-N-glutamate isomer) and SA [68] (2). A large proportion of SA is glucosylated to SA-β-D-glucoside (labelled SA-glucose) and a smaller proportion to the glucose-SA ester and both of these biologically inactive molecules accumulate in the vacuole and may act as stores or reserves of SA. SA can also be metabolized to various dihydroxybenzoates, which can also be glycosylated (omitted here for simplicity). Methyl-SA is volatile and can act as a resistance inducer and also influences plant-insect interactions. **(b)** In bacteria SA, which is typically utilized for the synthesis of siderophores, is derived from the shikimate pathway. In some bacteria (e.g. *Pseudomonas aeruginosa*), chorismate is converted to SA via isochorismate by two enzymes, ICS and IPL **(i)**. In others (e.g. *Yersinia*

657 *enterocolitica*) an SA synthase, i.e. a bifunctional enzyme with both ICS and IPL
658 activity, converts chorismate directly to SA (shown with isochorismate as a transient
659 intermediate) **(ii)**. Abbreviations: AO4, aldehyde oxidase 4; BA, benzoic acid; BA2H,
660 BA 2-hydroxylase; BSMT, BA/SA carboxyl methyltransferase; 4-CL, 4-
661 coumarate:CoA ligase; EDS5, Enhanced Disease Susceptibility 5 (isochorismate
662 transporter); EPS1, a member of the BAHD acyltransferase protein family; ICS,
663 isochorismate synthase; IPL, isochorismate pyruvate-lyase; PAL, phenylalanine
664 ammonia-lyase; PBS3, avrPphB SUSCEPTIBLE 3 (an amidotransferase), and SA,
665 salicylic acid. Based on references [17,18,24,25,27**,68].

666

667 **Figure 2. Salicylic acid sits at the center of a complex network regulating**
668 **resistance to viruses and other pathogens**. The diagram depicts in simplified form
669 some of the SA-dependent resistance phenomena described in this article (blue-
670 outlined boxes). SA can have direct effects on antiviral defense (pale blue arrows)
671 through its effects on ROS generation in mitochondria or its inhibitory effect on
672 GAPDH (a component of tombusviral replicase complexes). SA-induced ROS
673 increases in the mitochondria result in increased resistance to viruses and AOX
674 activity and glutathione levels modulate this form of signaling. SA can also stimulate
675 resistance to viral intercellular movement via a less well-characterized AOX-
676 independent signaling system (dark blue arrow). Working through the master
677 regulatory factor NPR1 (and its partners NPR3 and 4 and TGA transcription factors,
678 which are omitted for simplicity) SA stimulates the transcription of PR mRNAs,
679 contributing to defense against non-viral pathogens. SA-stimulated increases in
680 RDR1 transcription (and possibly SA-stimulated increases in RDR1 activity) are also
681 dependent on NPR1. RDR1 also influences transcription of *RDR6* and *AOX*
682 (indicated by asterisks). Abbreviations: AOX, alternative oxidase; GAPDH,
683 glyceraldehyde 3-phosphate dehydrogenase; NPR1, Non-Expresser of PR proteins
684 1; PR, pathogenesis-related protein; RDR, RNA-dependent RNA polymerase, and
685 ROS, reactive oxygen species. Based on references [2,23,38*,41,44,45,49,50**,51].

686

687

688 **Figure 3. Salicylic acid as anti-viral or pro-viral factor**. **(a)** In plants possessing a
689 dominant virus-specific resistance (*R*) gene, recognition of virus (depicted in this
690 cartoon by an icosahedron) triggers a hypersensitive reaction (HR), a resistance
691 response in which localization of the invading virus to the vicinity of the inoculation
692 site is dependent in part upon rapid production of salicylic acid by the host (SA). In
693 susceptible plants that have been treated with exogenous SA, the spread of virus out
694 of the inoculation zone is inhibited but not always completely halted. **(b)** Certain
695 viruses (e.g. potyviruses, cauliflower mosaic virus, cucumber mosaic virus, and
696 tobacco rattle virus) stimulate endogenous SA biosynthesis as they spread
697 systemically through susceptible hosts, which limits virus accumulation and
698 ameliorates disease symptoms. **(c)** In plants depleted in SA (by transgenic
699 expression of SA-hydroxylase, or in mutant plants lacking SA biosynthetic capacity)
700 virus accumulation is enhance but this may lead to severe stunting of plants (and an
701 overall decrease in virus yield per plant) or symptoms may be exacerbated leading
702 to necrosis (likely leading to inactivation of virus particles present in the necrotizing
703 tissue). Thus, in scenario **(b)**, the virus is exploiting SA as a pro-viral factor by
704 ensuring that virus accumulation is optimized. Based on references
705 [1,2,4,5,35,53,63*].

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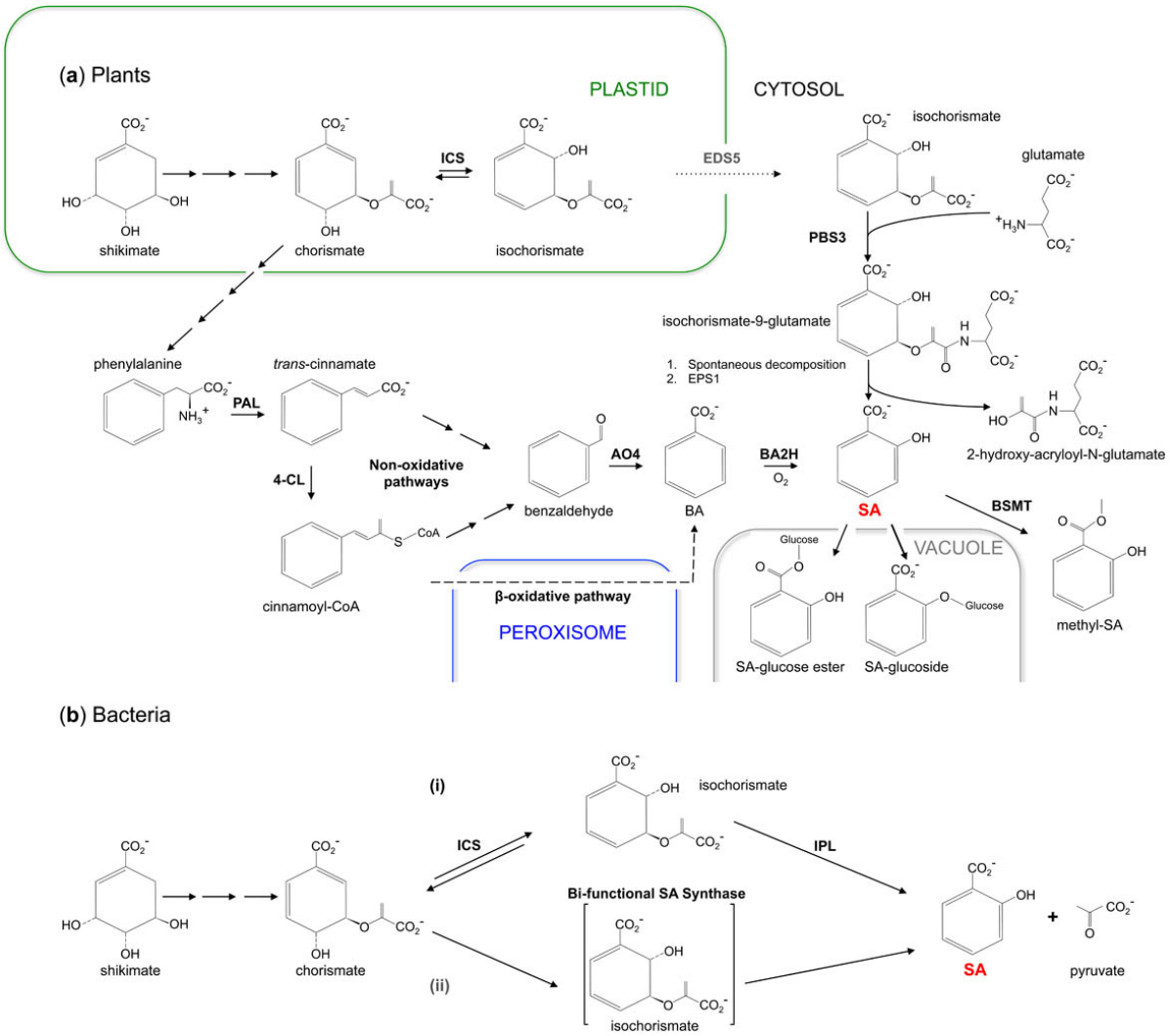

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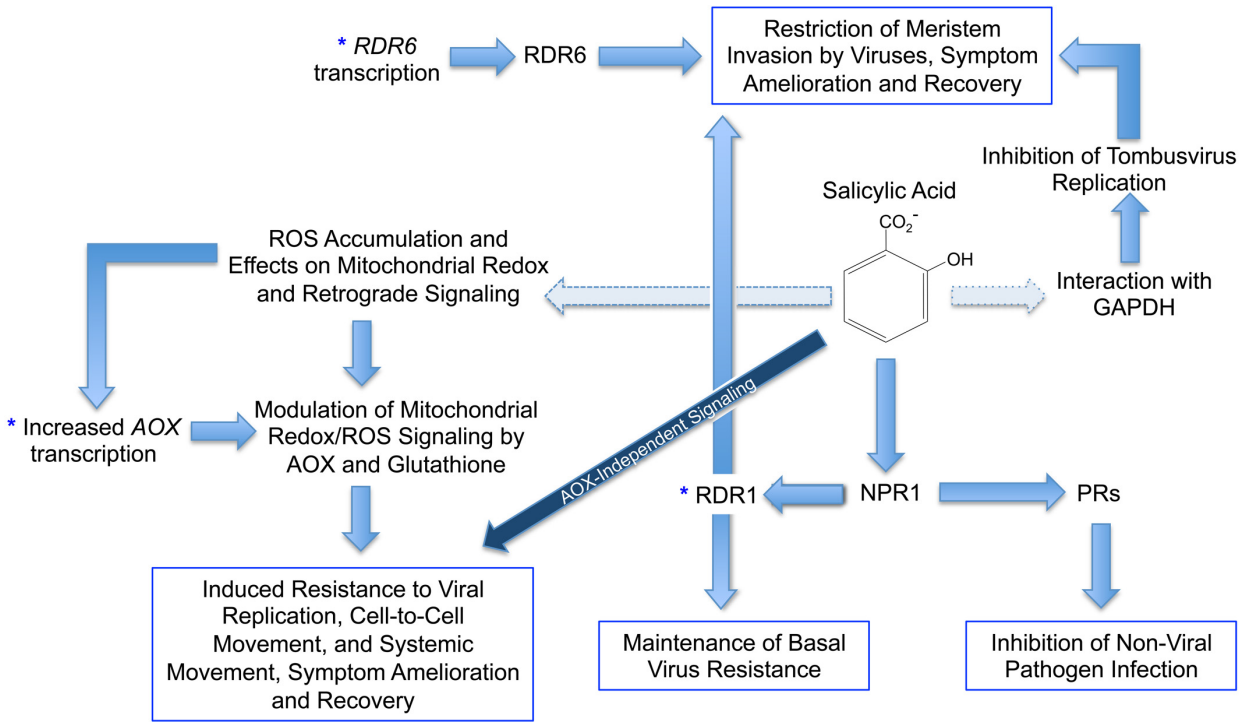
* RDR6 transcription
RDR6
Restriction of Meristem Invasion by Viruses, Symptom Amelioration and Recovery
Inhibition of Tombusvirus Replication
Salicylic Acid
CO2-
OH
Interaction with GAPDH
ROS Accumulation and Effects on Mitochondrial Redox and Retrograde Signaling
* Increased AOX transcription
Modulation of Mitochondrial Redox/ROS Signaling by AOX and Glutathione
AOX-Independent Signaling
* RDR1
NPR1
PRs
Induced Resistance to Viral Replication, Cell-to-Cell Movement, and Systemic Movement, Symptom Amelioration and Recovery
Maintenance of Basal Virus Resistance
Inhibition of Non-Viral Pathogen Infection

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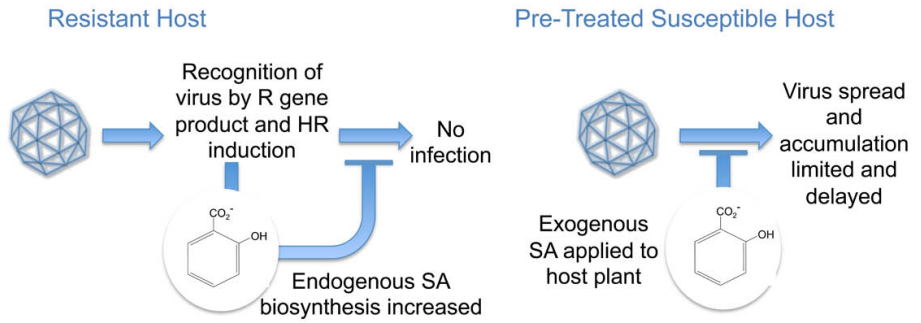
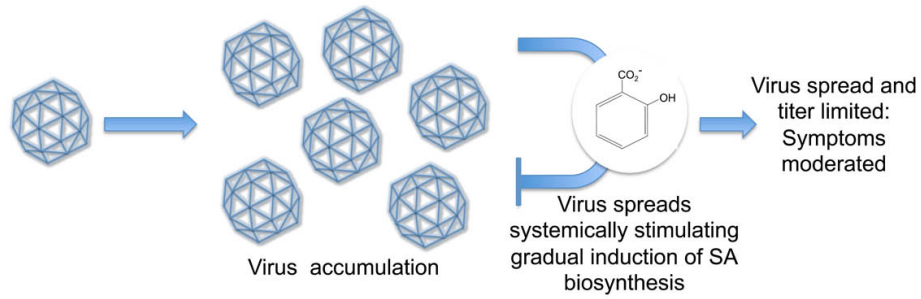
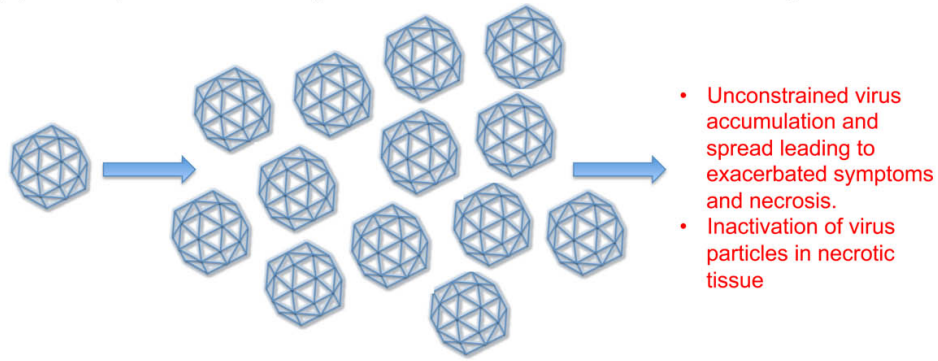
(a) Resistant host or susceptible host pre-treated with SA



(b) Susceptible host



(c) Susceptible host compromised in SA accumulation or biosynthesis



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