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Article Addendum

The search for the salicylic acid receptor led to discovery of the SAR signal receptor

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Systemic acquired resistance (SAR) is a state of heightened defense which is induced throughout a plant by an initial infection; it provides long-lasting, broad-spectrum resistance to subsequent pathogen challenge. Recently we identified a phloem-mobile signal for SAR which has been elusive for almost 30 years. It is methyl salicylate (MeSA), an inactive derivative of the defense hormone, salicylic acid (SA). This discovery resulted from extensive characterization of SA-binding protein 2 (SABP2), a protein whose high affinity for SA and extremely low abundance suggested that it might be the SA receptor. Instead we discovered that SABP2 is a MeSA esterase whose function is to convert biologically inactive MeSA in the systemic tissue to active SA. The accumulated SA then activates or primes defenses leading to SAR. SABP2's esterase activity is inhibited in the initially/primary infected tissue by SA binding in its active site; this facilitates accumulation of MeSA, which is then translocated through the phloem to systemic tissue for perception and processing by SABP2 to SA. Thus, while SABP2 is not the SA receptor, it can be considered the receptor for the SAR signal. This study of SABPs not only illustrates the unexpected nature of scientific discoveries, but also underscores the need to use biochemical approaches in addition to genetics to address complex biological processes, such as disease resistance.

For over a century, naturalists and scientists have observed that plants which survive an initial pathogen attack often develop enhanced resistance to subsequent infections. Systematic studies by Frank Ross in the early 1960s demonstrated that prior infection of tobacco plants by Tobacco Mosaic Virus (TMV) enhanced resistance in the systemic tissue to subsequent challenge by TMV or other pathogens, which he termed systemic acquired resistance.[1] In the later 1970s Kuc and others showed that development of SAR required movement of a signal made in the primary infected tissue through the phloem to the distal systemic tissue.[2]

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More recent studies starting in 1990 indicated that SA plays a critical role(s) in plant disease resistance.[3-5] For the past decade and a half we have used biochemical and genetic approaches to identify the components and molecular mechanisms involved in SA-mediated signal transduction. One approach was to biochemically identify proteins in tobacco which bound SA, with the hope that some would be SA effectors or targets and at least one would be a receptor for SA. This led to the identification of catalase, ascorbate peroxidase, SABP3, which is the chloroplastic carbonic anhydrase, and SABP2.[6-9] SA inhibits catalase's and ascorbate peroxidase's H_2O_2 scavenging activities; this inhibition may contribute to the oxidative burst that occurs after infection by avirulent microbes and the subsequent alteration in cellular redox state that facilitates relocation of the positive regulator protein NPR1 from the cytoplasm to the nucleus for activation of SA-responsive defense genes, such as *PR-1*.[5,10] While SA does not appear to alter carbonic anhydrase's activity, altering carbonic anhydrase synthesis suppressed defense responses and/or disease resistance.[9,11,12]

SABP2 is a very low abundance (10 fmol/mg), soluble protein of ~30 kDa that exhibits high affinity for SA (K_d = 90 nM).[7] Because these properties suggested that SABP2 might be an SA receptor, we spent five years and overcame several setbacks to purify this protein and clone its gene.[13] One major setback was a dramatic reduction and eventual discontinuation of funding by the National Science Foundation (NSF). This discontinuation reflected in part the historically low grant funding levels at U.S. government agencies due to the Iraq war. Another obstacle was the prevailing attitude that biochemical approaches were inefficient/ineffective. Indeed, some of the grant reviewers questioned why we were wasting our time using such a challenging approach when genetics would eventually reveal SABP2 function. Despite these obstacles, we succeeded in partially purifying SABP2, cloning its gene, and demonstrating that SABP2 has esterase/lipase activity and is involved in disease resistance, including SAR.[13]

The second major breakthrough on the SABP2 project involved using a combination of biochemistry, enzymology and biophysics. X-ray crystallography revealed that SA was bound in SABP2's active site; this suggested that SA binding would lead to inhibition of SABP2's esterase activity as its active site is too small to accommodate both its substrate and SA. Biochemical analyses confirmed this hypothesis and also established that MeSA is SABP2's likely substrate.[14] Subsequent studies confirmed that MeSA is SABP2's

in planta substrate, while grafting experiments revealed that SABP2 is required in the systemic tissue for perception/processing of the SAR signal but not in the primary infected tissue for generation of the SAR signal.[15] Structure-function analyses, based on SABP2's enzymology and 3-D structure in complex with SA, revealed that SABP2's MeSA esterase activity is required in the systemic tissue while SABP2's SA-binding activity and the resulting feedback inhibition of its MeSA esterase activity are needed in the primary infected tissue for an effective SAR response. Together these results argued that MeSA is the long-sought mobile SAR signal. This was confirmed by quantification of MeSA and SA in the primary infected tissue, in phloem exudates from this tissue and in the systemic tissue of wild type and SAR-deficient mutant or transgenic plants. This conclusion was further supported by the demonstration (via RNAi-mediated gene silencing) that the enzyme responsible for MeSA production from SA, SA methyl transferase, is required in the SAR signal-generating, primary infected tissue, but not in the systemic tissue.[15]

Subsequent analyses strongly suggests that MeSA also is an SAR signal in Arabidopsis[16] and potato (Manosalva P, Park SW, Klessig DF, unpublished results). Since Arabidopsis contains five MeSA esterases and most of these must be silenced in order to inhibit SAR development,[16] classical genetic analyses did not and would not have revealed the role of these genes in SAR. In sum, the results of this 15 plus year project illustrate that persistence, even in the face of adversity, may be necessary to succeed, and it can pay off in rather unexpected ways. Our results also demonstrate that it is important to use biochemical and biophysical approaches, in combination with genetics, to explore complex biological processes.

References

1. Ross AF. Systemic acquired resistance induced by localized virus infections in plants. Virology 1961; 14:340-58.
2. Jenns A, Kuc J. Graft transmission of systemic resistance of cucumber to anthracnose induced by *Colletorichum lagenarium* and tobacco necrosis virus. Phytopathology 1979; 69:753-6.
3. Malamy J, Carr JP, Klessig DF, Raskin I. Salicylic acid—a likely endogenous signal in the resistance response of tobacco to tobacco mosaic virus infection. Science 1990; 250:1002-4.
4. Métraux JP, Signer H, Ryals J, Ward E, Wyss Benz M, Gaudin J, Raschdorf K, Blum W, Inverardi B. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science 1990; 250:1004-6
5. Dempsey D, Shah J, Klessig DF. Salicylic acid and disease resistance in plants. Crit Rev Plant Sci 1999; 18:547-75.
6. Chen Z, Silva H, Klessig DF. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science 1993; 262:1883-6.
7. Du H, Klessig DF. Identification of a soluble, high affinity salicylic acid-binding protein in tobacco. Plant Physiol 1997; 113:1319-27.
8. Durner J, Klessig DF. Inhibition of ascorbate peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense responses. Proc Natl Acad Sci USA 1995; 92:11312-6.
9. Slaymaker DH, Navarre DA, Clark D, del Pozo O, Martin GB, Klessig DF. The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. Proc Natl Acad Sci USA 2002; 99:11640-5.
10. Mou Z, Fan W, Dong X. Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell 2003; 113:935-44.
11. Clark D, Rowlett RS, Coleman JR, Klessig DF. Complementation of the yeast deletion mutant ΔNCE103 by members of the β class of carbonic anhydrases is dependent on carbonic anhydrase activity rather than on antioxidant activity Biochem J 2004; 379:609-15.
12. Restrepo S, Myers KL, del Pozo O, Martin GB, Hart AL, Buell CR, Fry WE, Smart CD. Gene profiling of a compatible interaction between *Phytophthora infestans* and *Solanum tuberosum* suggests a role for carbonic anhydrase. Mol Plant-Microbe Interact 2005; 18:913-22.
13. Kumar D, Klessig DF. The high affinity salicylic acid binding protein (SABP2) is required for plant innate immunity and has SA-stimulated lipase activity. Proc Natl Acad Sci USA 2003; 100:16101-6.
14. Forouhar F, Yang Y, Kumar D, Chen Y, Fridman E, Park SW, Chiang Y, Acton TB, Montelione GT, Pichersky E, Klessig DF, Tong L. Crystal structure and biochemical studies identify tobacco SABP2 as a methylsalicylate esterase and further implicate it in plant innate immunity. Proc Natl Acad Sci USA 2005; 102:1773-8.
15. Park SW, Kaiyomo E, Kumar D, Mosher SL, Klessig DF. Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 2007; 318:113-6.
16. Vlot AC, Liu PP, Cameron RK, Park SW, Yang Y, Kumar D, Zhou F, Padukkavidana T, Gustafsson C, Pichersky E, Klessig DF. Identification of orthologs of tobacco salicylic acid-binding protein 2 and their role in systemic acquired resistance in *Arabidopsis thaliana*. Plant J 2008; In review.