

Mini-Review Open Access

The Road to RNA Silencing is Paved with Plant-Virus Interactions

Peter Palukaitis*

Department of Horticultural Sciences, Seoul Women's University, Seoul 139-774, Korea

(Received on May 11, 2011; Accepted on May 24, 2011)

RNA silencing has had a large impact on biology in general, as well as on our understanding of plant-pathogen interactions, especially interactions between plants and viruses. While most of what we know about the mechanism of RNA silencing was deduced in the last 12 years, many of the interactions between plants and viruses, as well as virus-virus interactions in plants, which we now know are manifestations of RNA silencing, were the subject of decades of work from numerous laboratories. These laboratories were examining the nature and extent of phenomena such as recovery from infection, the formation of dark green islands resistant to re-infection, synergy between unrelated viruses and cross-protection between related viruses, all first described in the late 1920s. In this review, the relationships between these phenomena and their place in the defense mechanism we call RNA silencing will be described, to show how they are all linked.

Keywords : RNA silencing, recovery, dark-green islands, cross-protection, synergy, RNA silencing suppressors

RNA silencing or RNA interference (RNAi) in animals is a system for regulation of ectopic gene expression, post-transcriptional RNA turnover, removal of aberrant RNAs, silencing of retrotransposons, and defense against virus infection (Brodersen and Voinnet, 2006; Carr et al., 2010; Chitwood and Timmermans, 2010; Csorba et al., 2009; Ding and Voinnet, 2007; Eamens et al., 2008; Lewsey et al., 2009; Moazed, 2009; Li and Ding, 2006; van Rij and Andino, 2006; Vaucheret, 2006; Voinnet, 2005a, b). This process regulates organismal development as well as the normal maintenance of homeostatic gene functions. Although the research that led to the discovery of RNA silencing by double-stranded (ds) RNA, culminating in the Nobel Prize in Physiology and Medicine being awarded in 2006 to Fire and Mello, was developed first in a free-living nematode (Fire et al., 1998), the phenomenon of RNA silencing itself was known some years earlier in plants starting with the discovery that transgenic sense and anti-

sense RNAs could both suppress the expression of a related gene (Jorgensen et al., 1996; van der Krol et al., 1990; Napoli et al., 1990; Smith et al., 1990). Subsequent work in plants led to the idea that there was some sort of plant surveillance system which could degrade RNAs, producing small RNAs, and that the plant-encoded RNA-dependent RNA polymerase (RdRp) possibly was involved in this process (Goodwin et al., 1996; Lindbo et al., 1993). In the following years, small RNAs of 21–25 nt were found associated with degradation of transgene RNAs (Hamilton and Baulcombe, 1999; Mette et al., 2000) and the tomato RdRp gene (RDR) was isolated and characterized (Schiebel et al., 1998). Soon thereafter, genes in a bread mold (Cagoni and Machino, 1999), a nematode (Schmardon et al., 2000) and a plant (thale cress; Dalmay et al., 2000; Mourrain et al., 2000) involved in RNA silencing all were found to be similar to the tomato RDR gene. Later, it was shown that while there was only one RDR gene in lower eukaryotes, there were six RDR genes in *Arabidopsis thaliana* and that different RDRs were involved in either overlapping or distinct pathways in RNA silencing (Chan et al., 2004; Garcia-Ruiz et al., 2010; Qu et al., 2005; Schwach et al., 2005; Wang et al., 2010; Yang et al., 2004; Yu et al., 2003). Thus, it became clear that plants, fungi and (some) animals all contained a novel RNA degradation system that regulated gene expression. In congruence with the discovery that plant viruses encoded suppression proteins of this cell surveillance system (Csorba et al., 2009), it was apparent that this RNA degradation system also was involved as a novel virus resistance mechanism distinct from the innate immunity system common to resistance to various pathogens in plants (Chisholm et al., 2006). While many details of the components of this system were first described in animal models (Dicer, RISC, Argonaute-like proteins, miRNAs, etc.), further work in plants complemented the work in animal models and extended them. This was due largely to the number of orthologous genes involved in overlapping or unique aspects the RNA silencing pathway, the wealth of mutants affecting various steps in RNA silencing in *A. thaliana*, and the plethora of plant virus-encoded suppressors of RNA silencing (VSR), which delimited and refined the mechanism of the RNA silencing pathway. Similarly, while practical applications of RNA

*Corresponding author.

Phone) +82-2-970-5614, FAX) +82-2-970-5610

E-mail) peterp@swu.ac.kr

silencing in medicine have not yet become manifest, they have been utilized effectively in plant systems in agriculture (Gottula and Fuchs, 2009).

Although RNA silencing and various related aspects, such as ectopic gene regulation, are at the forefront of molecular biology, many of the manifestations of RNA silencing were known for many decades, although their significance and mechanistic details were obscure. In epistemological terms, they were *unknown knowns*; i.e., something we knew of, but did not understand where it fit or what it meant. This mini-review will recapitulate these early phenomena associated with plant virus interactions in plants, showing how they are associated with various aspects of RNA silencing and its suppression.

Recovery

The description by Wingard (1928) of the course of infection of the nepovirus tobacco ringspot virus, included the observations that after some time, the new leaves that emerged were symptomless and also were resistant to re-infection by the same virus. Similar observations were made over the intervening decades with alfalfa mosaic virus (Ross, 1941), other nepoviruses (Harrison, 1958; Lister and Murrant, 1967), tobacco rattle virus (TRV; Cadman and Harrison, 1959) and cauliflower mosaic virus (CaMV) in kohlrabi (Al-Kaff and Covey, 1995). In addition, except for CaMV, these other viruses also were all seed transmissible to some extent (Mink, 1994), indicating a link between the recovery state and the ability to enter the meristem. However, the nature of the recovery state remained a mystery until the late 1990s. The seminal work that explained the nature of the recovery state was preceded by observations from some lines of transgenic plants expressing viral sequences. It was observed that plants expressing moderate-to-high levels of the viral transgene would initially become infected by the corresponding virus, but then these plants would recover from infection at which point they also had no detectable virus in the upper leaves and suppressed accumulation of the transgene RNA (Lindbo et al., 1993; Smith et al., 1994). Those authors suggested that such transgenic plants may require infection of the apical meristem to bring about this recovery state, which was caused by activation of the RNA degradation system (Lindbo et al., 1993). Following on from this work, Covey et al. (1997) and Ratcliff et al. (1997) independently showed a linkage between recovery and what we now call RNA silencing. Covey et al. (1997) showed that the CaMV 35S and 19S RNAs were degraded in recovered kohlrabi plants, while host RNAs were not affected, demonstrating that the effects observed in virus-infected, recovering transgenic plants also occurred in plants that were recovering from

virus infection but in the absence of a viral transgene. This indicated that the two types of recovery probably were related, mechanistically. Ratcliff et al. (1997) re-examined the recovery observed during infection by a nepovirus, tomato black ring virus, and the ability of such recovered plants to resist re-infection. They showed that the recovered tissue did contain low levels of virus, but could not be re-infected by the same virus or a closely related strain of the same virus, yet these tissues could be infected by a different nepovirus or an unrelated virus, potato virus X (PVX). In addition, such recovered plants also could not be re-infected by a PVX vector containing sequences of tomato black ring virus, indicating that the mechanism preventing replication of the second virus was based on the relatedness of the RNA sequences of the two viruses. A similar approach was then used to show that plant tissue that had recovered from infection by TRV expressing the green fluorescent protein (GFP) was immune to re-infection by PVX expressing the enzyme β -glucuronidase (GUS) and part of the GFP gene, but not to PVX-GUS alone (Ratcliff et al., 1999). In a quite different situation, after infection by cucumber mosaic virus (CMV), the plants showed only a transient recovery leading to a cycling of symptoms in tobacco (Gal-On et al., 1995; Loebenstein et al., 1977) and *Nicotiana benthamiana* (Fukuzawa et al., 2010). Interestingly, in *N. benthamiana* (Fukuzawa et al., 2010), but not tobacco (Shams-Bakhsh et al., 2007), transgenic expression of HC-Pro, a VSR from potato virus Y (PVY), prevented the cycling of symptoms induced by CMV. Unlike tobacco (Xie et al., 2001), *N. benthamiana* does not contain a functional RDR1 (Yang et al., 2004). These experiments all demonstrated that recovery was a manifestation of RNA silencing.

What exactly initiates recovery in such plants remains unclear, but it does appear to relate to the invasion of the meristem by the virus at some stage during infection, and shortly thereafter RNA silencing leading to recovery is activated. Prior to this step, the virus is able to evade or suppress the RNA silencing mechanism. Apparently, the ability of RNA to enter the meristem is compromised by several VSR (Foster et al., 2002; Martínez-Hernández and Baulcombe, 2008; Sunpapao et al., 2009) and in some cases, RDR6 is involved in preventing RNAs from entering the meristem (Di Serio et al., 2010; Qu et al., 2005; Schwach et al., 2005). Additionally, in some cases, the level of RDR6 mRNA was down-regulated by the action of VSR (Zhang et al., 2008). Similarly, seed transmission, which is linked to entry of viruses into the meristem, has been mapped in some cases to viral genes now known to be VSR (Csorba et al., 2009; Edwards, 1995; Johansen et al., 1996; Maule and Wang, 1996; Wang et al., 1997). These studies all provide further links between recovery, entering the meristem, seed transmission, and the RNA silencing system.

Synergy

In many cases, infection by unrelated viruses led to an interaction called synergy, in which the disease produced was worse than caused by either virus alone (Palukaitis and MacFarlane, 2006). Such synergistic interactions were first described in the 1920's, by Vanterpool (1926), Blood (1928), and Smith (1928). More than 20 examples of synergistic interactions have been described since then, most, but not all of which, involved a potyvirus or a tobamovirus, which usually did not increase in accumulation (Palukaitis and MacFarlane, 2006). However, in almost all examples so characterized, the other virus in the mixture showed an increase in virus accumulation; while in some rare cases either both viruses or neither virus increased in accumulation (Palukaitis and Kaplan, 1997; Palukaitis and MacFarlane, 2006). Much detailed work was done by Ross and colleagues between 1950 and 1974, characterizing the nature of the synergistic interactions between PVX and PVY. They showed that co-infection by PVX and PVY resulted in an increase in accumulation of PVX, but not of PVY, and that co-inoculation of the two viruses resulted in higher levels of PVX accumulation than when either virus was inoculated some days before the other (Rochow and Ross, 1955; Goodman and Ross, 1974a). Their data suggested that for PVX levels to increase, the cells of the infected leaves needed to be doubly infected with both PVX and PVY (Goodman and Ross, 1974b), and these cells needed to be actively accumulating PVY when PVX infection took place (Damirdagh and Ross, 1967; Goodman and Ross, 1974a). This was also shown to be the case for the synergistic interactions between the potyvirus zucchini yellow mosaic virus and the cucumovirus CMV (Poolpol and Inouye, 1986). The increase in PVX accumulation paralleled the increase in disease synergy, which was dependent on a number of factors such as the temperature, the type of tissue analyzed, and the growth stage of the inoculated plant (Rochow and Ross, 1955). Damirdagh and Ross (1967) speculated that "infection by PVX normally induces reactions that act to limit PVX synthesis but is unable to do so in a cell in which PVY is actively multiplying." The nature of the substance limiting PVX infection was not understood, but we now recognize this as RNA silencing.

Little further was done mechanistically concerning the nature of synergy, until Vance (1991) re-examined the interactions between PVX and PVY, using molecular tools not available in the earlier studies. Vance confirmed and extended the conclusions of Goodman and Ross (1974a, b), showing that both the level of PVX RNA and coat protein (CP) increased during the synergistic interaction with PVY, but neither component of PVY showed an increase in

accumulation. She also showed that during the synergistic interaction, the (-) RNA of PVX increased disproportionately (~8 fold) in comparison with the increase in (+) PVX RNA observed in the doubly infected plants (Vance, 1991). This increase in accumulation of (-) RNA, which is usually found in the form of dsRNAs after isolation, is consistent with synergy affecting some process that reduces the accumulation of dsRNA, although this may be a simplistic explanation, since such disproportional increases were not always observed and appeared to depend upon both the host used and the particular virus combinations tested (García-Cano et al., 2006; González-Jara et al., 2004; Wang et al., 2002). In some cases, synergy between viruses could also break resistance plants exhibited against one of the viruses (Choi et al., 2002; Murphy and Kyle, 1995; Wang et al., 2004), or transiently enhance the spread of another virus (Sáenz et al., 2002). Whether the nature of these resistance responses were manifestations of RNA silencing is not known.

By the mid-1990's, several laboratories has produced transgenic plants expressing various potyviral sequences. Vance and her colleagues took advantage of this to take our understanding of synergy to the next level. After showing that other potyviruses [tobacco vein mottling virus and tobacco etch virus (TEV)] could interact synergistically with PVX in tobacco, Vance et al. (1995) showed that some transgenes derived from these viruses could increase both PVX-induced pathogenicity and also the accumulation of PVX in transgenic tobacco. Specifically, PVX accumulation and virulence was increased in transgenic tobacco plants expressing the P1/HC-Pro/(partial or complete) P3 encoding regions of these two potyviruses, but not in transgenic tobacco expressing other coding sequences of tobacco vein mottling virus (Vance et al., 1995). Mutational analysis showed that the central region of the HC-Pro encoding sequences was involved in this activity (Shi et al., 1995). They extended their work to show that transgenic plants expressing the P1/HC-Pro/(partial) P3 coding sequences of TEV could also stimulate enhanced pathogenicity and virus accumulation after infection by tobacco mosaic virus (TMV) or CMV (Pruss et al., 1997); two other viruses which also interacted synergistically with TEV. This demonstrated that this phenomenon was not limited to PVX and potyviruses, and that synergy did not require the replication of both viruses, but rather specific sequences. Using a PVX vector to express TEV sequences, Pruss et al. (1997) also showed that the specific viral sequence required to enhance pathogenicity and the increase in accumulation of both (+) and (-) PVX RNA was the P1/HC-Pro of TEV, and that it was the protein and not just the RNA sequence encoding the P1/HC-Pro that caused this effect; HC-Pro alone also increased PVX RNA accumulation, but not to the same extent as P1/

HC-Pro (Pruss et al., 1997). This may be some due to some role of the P1 protein in the optimal folding of the HC-Pro during translation. Thus, the stage was set for the next step, to determine what the function of P1/HC-Pro was in enhancing accumulation and virulence of unrelated viruses; an epistemological *known unknown*.

RNA Silencing Suppressors

In late 1998, three papers appeared also showing that the HC-Pro was a suppressor of the RNA silencing of a reporter transgene (Anandalakshmi et al., 1998; Brigneti et al., 1998; Kasschau and Carrington, 1998), indicating that gene silencing was also a natural antiviral defense system. Anandalakshmi et al. (1998) showed that the crosses made between transgenic tobacco plants expressing the TEV P1/HC-Pro/(partial) P3 and transgenic plants silenced for GUS expression resulted in progeny plants that could express GUS. Such plants were also susceptible to infection by PVX expressing GUS. Moreover, while infection of GFP-expressing plants by PVX expressing GFP would induce the silencing of expression of GFP and ultimately inhibit PVX accumulation [the so-called virus-induced gene silencing (VIGS; Ruiz et al., 1998)], PVX expressing P1/HC-Pro and GFP did not induce VIGS and PVX expressing either P1/HC-Pro or just HC-Pro could interfere with the PVX-GFP-induced VIGS in co-inoculated GFP-expressing transgenic plants. Kasschau and Carrington (1998) also used transgenic plants silenced for expression of a defective GUS gene to show that these could not be infected by PVX-GUS, but could be infected by PVX-GFP, and that if such plants were crossed with transgenic plants expressing P1/HC-Pro of TEV, the segregating progeny could be infected by PVX-GUS, if they contained both GUS and P1/HC-Pro genes, or the P1/HC-Pro gene alone, but not the GUS gene alone. Moreover, those plants containing both P1/HC-Pro and GUS genes showed high levels of GUS mRNA accumulation, unlike the lines without the P1/HC-Pro gene, which accumulated low levels of GUS mRNA, due to RNA silencing. Brigneti et al. (1998) used GFP-expressing plants that were silenced by agroinfiltration and then showed that both PVY and CMV could reverse the silencing. In addition, they showed that the HC-Pro of PVY and the 2b gene of CMV could also reverse the silencing of the GFP transgene, when expressed from a PVX vector. Interestingly, they found that while the HC-Pro could affect the maintenance of RNA silencing, by reversing RNA silencing in leaves in which it has become established, the CMV 2b protein could not do so, and only prevented RNA silencing from becoming established in new leaves that had not emerged at the time of inoculation. This showed that the two VSR had different effects and presumably different

targets.

Over the following years, viruses belonging to most plant virus genera (Carr et al., 2010; Csorba et al., 2009; Ding and Voinnet, 2007; Lewsey et al., 2009; Li and Ding, 2006; Palukaitis and MacFarlane, 2006) and some animal virus genera (Bivalkar-Mehla et al., 2011) were shown to contain VSR, and in some cases, the specific targets or roles of these VSR also have been deduced. The various VSR do not have common sequences or structural motifs/domains and most of them have other roles in the virus infection cycle, including in replication, movement, encapsidation and transmission. Some VSR have common targets in the RNA silencing pathway (Carr et al., 2010; Csorba et al., 2009; Ding and Voinnet, 2007; Lewsey et al., 2009), although most, if not all, bind siRNAs (Lakatos et al., 2006; Mérei et al., 2006; Rashid et al., 2008). Some viruses have more than one VSR. The closterovirus citrus tristeza virus contains three VSR, with different specificities: the P23 inhibits intracellular silencing, the CP inhibits long-distance signaling and the P20 does both (Lu et al., 2004). The crinivirus tomato chlorosis virus also contains three VSR: the P22 encoded by RNA 1 interfered with local RNA silencing but not with the spread of the silencing signal, while both the major and minor capsid proteins, encoded by RNA 2, also showed local RNA silencing suppression activity (Cañizares et al., 2008). On the other hand, the crinivirus sweet potato chlorotic stunt virus expresses a small dsRNase that cleaves siRNAs such that they cannot function in RNA silencing and works in concert with the P22 protein of this virus (Cuellar et al., 2006; Kreuze et al., 2005). Thus, even between viruses within the same genus there are differences in the functions of VSR. Differences in levels of virus accumulation between hosts (and viruses) may reflect the extent to which a VSR is able to suppress the host defense against that virus. Therefore, it is not surprising that when two viruses are present in a mixed infection and they have different targets of inhibition of the RNA silencing pathway, at least one virus show an increase in virus accumulation.

It is likely that virtually all viruses contain some sort of VSR, with the likely exception of cryptic viruses. These are dsRNA viruses that apparently only express genes encoding a replicase and a CP, and replicate inside the virus particles (Boccardo and Accotto, 1988). They are not infectious to plants, but are present at low levels in all cells of plants containing them, being transmitted only vertically through seeds (Boccardo et al., 1987).

Dark Green Islands

The infection of many plants by viruses leads to a mosaic symptom, in which light green or yellow areas are inter-

persed with dark green islands (DGIs). This symptom response was known before the concept of viruses was elucidated by Beijerinck in 1898 and was described for tobacco (Allard, 1914), although many other plant species infected by numerous viruses show this response. Holmes (1928) first showed that the DGIs contained less infective material than the chlorotic areas of tobacco leaves infected by TMV; he estimated the level of TMV present in DGIs at about 4% of the TMV present in the chlorotic tissue. In the 1960's and 1970's, other groups also found that the level of virus in the DGIs was 0–9% of that found in the chlorotic tissue, using different viruses and different hosts (rev. by Moore and MacDiarmid, 2006). More recently, using more sensitive molecular tools (nucleic acid hybridization) and larger numbers of samples, Moore et al. (2001) showed that the DGIs fell into three classes in *N. benthamiana* infected by the potyvirus, tamarillo mosaic virus (TaMV): 67% of the DGIs had less than 1% of the virus found in the yellow tissues; 19% of the DGIs had 1–10% of the virus present in the yellow areas; and 14% of the DGIs had 10–50% of the virus found in the yellow areas. The reasons for this variation are unknown, but may represent different extents of penetration of the tissue by an inhibitor. The nature of this inhibitor is likely to be RNA silencing.

As early as 1933, it was recognized that these DGIs were resistant to re-infection. Salaman (1933) showed that DGIs in plants infected by PVY were resistant to re-infection by the same or another strain of PVY. Others studies confirmed this observation, using different viruses. Fulton (1951) showed that DGIs of tobacco infected with TMV showed resistance to re-infection, Loebenstein et al. (1977) showed that the DGIs of tobacco infected with CMV were resistant to re-infection with CMV, but not with TMV, and Ferguson and Matthews (1993) showed that DGIs in Chinese cabbage infected with turnip yellow mosaic virus were resistant to re-infection by the same virus. Moore et al. (2001) also found that DGIs of *N. benthamiana* plants infected with the potyvirus TaMV were not resistant to infection by the potyvirus white clover mosaic virus, but were resistant to infection by a white clover mosaic virus vector expressing sequences of TaMV. In addition, transgenic plants expressing CP sequences of TaMV eventually recovered from infection by TaMV; however, the DGIs formed in infected leaves before recovery showed an absence of both virus accumulation and the accumulation of the TaMV transgene mRNA (Moore et al., 2001). These experiments demonstrated that DGIs are a manifestation of RNA silencing, although why they occur in such islands is not known. Interestingly, the formation of DGIs against a chimeric hordeivirus was either reduced or inhibited in transgenic *N. benthamiana* plants expressing a VSR, the HC-Pro of potato virus A (Yelina et al., 2002); however the DGIs

induced by infection with either TMV or CMV were not affected by the transgenic expression of PVY HC-Pro in tobacco (Shams-Bakhsh et al., 2007). Thus, DGI formation can be affected by VSR in some cases, as was the cycling of recovered and symptomatic tissues after infection by CMV (Fukuzawa et al., 2010), further linking these various phenomena involving plant-virus interactions.

Cross-protection

Cross-protection is a phenomenon occurring between related viruses, in which one virus interferes with or protects against infection by the other virus. Cross-protection was noted first by McKinney (1929), who observed the protection afforded by different strains of TMV against each other, when one strain was inoculated to the plant some days before the other strain. Similarly, Salaman (1933) observed that a mild strain of PVX could protect against infection by a severe strain of PVX in potato, and Webb et al. (1952) demonstrated that cross-protection also could occur between strains of the phloem-limited potato leafroll virus in potato. Many other examples of cross-protection have been described and it has been applied in the field since the early 1950's (Fulton, 1986; Gal-On and Shibolet, 2006; Ziebell and Carr, 2010). Since the 1930's, models to explain cross-protection have appeared, but it was not until the 1970's that testable models appeared. The earlier models included precursor exhaustion (the first virus using up some plant component that was required by a related virus) and filling of some cellular replication site (shared by related viruses, but not unrelated ones) (Ziebell and Carr, 2010). Later models included CP of the initial virus preventing uncoating of the (related) challenge virus (Sherwood and Fulton, 1982; de Zoeten and Fulton, 1975) and an RNA-mediated, RNA:RNA binding mechanism (Palukaitis and Zaitlin, 1984), to explain cross-protection involving viroids (Niblett et al., 1978), which have no CP (Ding, 2009; Flores et al., 2005). Various experiments indicated that CP was or was not necessary for cross-protection to occur between related viruses (Ziebell and Carr, 2010), without any firm resolution; those in favor of it and those against it are all convinced they are right! In an effort to demonstrate whether or not CP was involved in cross-protection, the Beachy group in collaboration with the Monsanto Company generated transgenic tobacco expressing TMV CP and showed that these plants were protected against infection by TMV, exhibiting a delay in systemic infection (Powell-Abel et al., 1986). Ironically, this was indeed a case of CP-mediated protection, rather than RNA-mediated resistance (Bendahmane and Beachy, 1999; Asurmendi et al., 2007), as was observed in most of the other studies using CP genes or other sequences of other viruses (Csorba et al., 2009).

However, given the subsequent work with transgenic plants expressing sequences of other viral genes and/or untranslatable sequences showing high resistance or a recovery form of resistance, doubt was again cast on the role of CP, or specifically, the need for CP, in cross-protection.

In examining whether “cross-protection” between unrelated viruses expressing common (GFP) sequences could also occur in non-recovered tissues, Ratcliff et al. (1999) showed that *N. benthamiana* plants systemically infected with PVX-GUS did not affect infection by TMV-GFP, but plants infected systemically by PVX-GUSGF (expressing GUS, but also containing part of the GFP gene), did prevent infection by TMV-GFP. Those authors concluded that this demonstrated that cross-protection was an RNA-mediated defense mechanism (Ratcliff et al., 1999). However, this does not explain the various instances of cross-protection, where CP was required (Powell-Abel et al., 1990; Sherwood and Fulton, 1982; Sherwood, 1987), or where using RNA as an inoculum could overcome cross-protection (Dodds et al., 1985; Nelson et al., 1987). Fulton (1986) also lists many cases of non-reciprocal cross-protection, for which there is no current explanation. These would have to be examined again, in the light of the current systems and technology available. With regard to RNA silencing as the sole mechanism, it should be pointed out that cross-protection between tobamoviruses in *Arabidopsis* did not require RDR6 activity (Kurihara and Watanabe, 2003) and cross-protection by CMV with a deletion of the 2b VSR against wild-type CMV did not require the Dicer-like RNases involved in siRNA-mediated protection (Ziebell and Carr, 1999). Thus, cross-protection does not involve a single mechanism, but as discussed by Gal-On and Shibboleth (1986) and Ziebell and Carr (2010) cross-protection is a phenomenon that has multiple mechanisms. For example, Culver (1996) observed good protection against infection by TMV by pre-inoculating the plants with PVX expressing the CP of TMV, whereas some protection, but not as good, was obtained when the PVX expressed non-translatable TMV CP sequences. Thus, RNA silencing and CP-mediated preventing of uncoating are likely to be two of the mechanisms of cross-protection, but there may be other underlying mechanisms not yet explored.

Conclusions

The pillars of RNA silencing rest on the foundations of various phenomena that were discovered in the late 1920's: recovery, synergy, DGIs and cross-protection. Recovery, DGIs and cross-protection (in part) are all manifestations of the same host defense response. The discovery of VSR came directly from identifying the role of specific gene products in viral synergism. Similarly, the identification of

RDR genes in RNA silencing followed on from work in the early 1970's showing that plants induced the expression of an RdRp upon infection (Fraenkel-Conrat, 1983; Wassenegger and Krczal, 2006). Some of these RdRps are now known to be involved not only in RNA silencing, but also in transcriptional silencing of DNAs and the epigenetic control of gene expression (Chan et al., 2004; Lippman and Martienssen, 2004; Wassenegger, 2005). RdRps are also involved in innate resistance, including the salicylic acid-mediated defense response (Gilliland et al., 2003; Xie et al., 2001; Yang et al., 2004) and the jasmonic acid-mediated defense response (Pandey and Baldwin, 2007; Pandey et al., 2008). Thus, the RdRps are key components of quite different defense pathways, providing defenses against viruses, bacteria, fungi and insects (Boller and Felix, 2009; Carr et al., 2010; Chisholm et al., 2006). It will be interesting to see what other phenomena or processes are linked to these various defense pathways. Perhaps an examination of the old literature for such phenomena will stimulate someone to make the next great breakthrough.

Acknowledgments

This study was supported by a special grant from Seoul Women's University in 2009.

References

- Al-Kaff, N. and Covey, S.N. Biological diversity of cauliflower mosaic virus isolates expressed from two Brassica species. *Plant Pathol.* 44:516–526.
- Allard, H. A. 1914. The mosaic disease of tobacco. *U. S. Dept. Agr. Bull.* 40:1–33.
- Anandalakshmi, R., Pruss, G. J., Ge, X., Marathe, R., Mallory, A. C., Smith, T. H. and Vance, V. B. 1998. A viral suppressor of gene silencing in plants. *Proc. Natl. Acad. Sci. USA* 95: 13079–13084.
- Asurmendi, S., Berg, R. H., Smith, T. J., Bendahmane, M. and Beachy, R. N. 2007. Aggregation of TMV CP plays a role in CP functions and in coat-protein-mediated resistance. *Virology* 366:98–106.
- Bendahmane, M. and Beachy, R. N. 1999. Control of tobamovirus infections via pathogen-derived resistance. *Adv. Virus Res.* 53:369–386.
- Bivalkar-Mehla, S., Vakharia, J., Mehla, R., Abreha, M., Kanwar, J. R., Tikoo, A. and Chauhan, A. 2011. Viral RNA silencing suppressors (RSS): novel strategy of viruses to ablate the host RNA interference (RNAi) defense system. *Virus Res.* 155:1–9.
- Blood, H. L. 1928. A “streak” of tomatoes produced by a disturbing principle by apparently healthy potatoes in combination with tomato mosaic virus. *Phytopathology* 18:311–315.
- Boccardo, G. and Accotto, G. P. 1988. RNA-dependent RNA polymerase activity in two morphologically different white

- clover cryptic viruses. *Virology* 163:413–419.
- Boccardo, G., Lisa, V., Luisoni, E. and Milne, R. G. 1987. Cryptic plant viruses. *Adv. Virus Res.* 32:171–214.
- Boller, T. and Felix, G. 2009. A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Ann. Rev. Plant Biol.* 60:379–406.
- Brigneti, G., Voinnet, O., Li, W.X., Ji, L. H., Ding, S. W. and Baulcombe, D. C. 1998. Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. *EMBO J.* 17:6739–6746.
- Brodersen, P. and Voinnet, O. 2006. The diversity of RNA silencing pathways in plants. *Trends Genet.* 22:268–280.
- Cadman, C. H. and Harrison, B. D. 1959. Studies on the properties of soil-borne viruses of the tobacco-rattle type occurring in Scotland. *Ann. Appl. Biol.* 47:542–556.
- Cañizares, M. C., Navas-Castillo, J. and Moriones, E. 2008. Multiple suppressors of RNA silencing encoded by both genomic RNAs of a crinivirus, *Tomato chlorosis virus*. *Virus Res.* 379:168–174.
- Carr, J. P., Lewsey, M. G. and Palukaitis, P. 2010. Signaling in induced resistance. *Adv. Virus Res.* 76:57–121.
- Chan, S. W.-L., Zilberman, D., Xie, Z., Johansen, L. K., Carrington, J. C. and Jacobsen, S. E. 2004. RNA silencing genes control de novo DNA methylation. *Science* 303:1336.
- Chisholm, S. T., Coaker, G., Day, B. and Staskawicz, B. J. 2006. Host-microbe interactions: Shaping the evolution of the plant immune response. *Cell* 124:803–814.
- Chitwood, D. H. and Timmermans, M. C. P. 2010. Small RNAs are on the move. *Nature* 467:415–419.
- Choi, S. K., Yoon, J. Y., Ryu, K. H., Choi, J. K., Palukaitis, P. and Park, W. M. 2002. Systemic movement of a movement-deficient strain of *Cucumber mosaic virus* in zucchini squash is facilitated by a cucurbit-infecting potyvirus. *J. Gen. Virol.* 83:3173–3178.
- Cogoni, C. and Macino G. 1999. Gene silencing in *Neurospora crassa* requires a protein homologous to RNA-dependent RNA polymerase. *Nature* 399:166–169.
- Covey, S. N., Al-Kaff, N. S., Lángara, A. and Turner, D. S. 1997. Plants combat infection by gene silencing. *Nature* 385:781–782.
- Csorba, T., Pantaleo, V. and Burgyán, J. 2009. RNA silencing: an antiviral mechanism. *Adv. Virus Res.* 75:35–71.
- Cuellar, W. J., Kreuze, J. F., Rajamäki, M.-L., Cruzado, K. R., Untiveros, M. and Valkonen, J. P. T. 2009. Elimination of antiviral defense by viral RNase III. *Proc. Natl. Acad. Sci. U.S.A.* 106:10354–10358.
- Dalmay, T., Hamilton, A., Rudd, S., Angell, S. and Baulcombe, D. C. 2000. An RNA-dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not a virus. *Cell* 101:543–553.
- Damirdagh, I. S. and Ross, A. F. 1967. A marked synergistic interaction of potato viruses X and Y in inoculated leaves of tobacco. *Virology* 31:296–307.
- de Zoeten, G. A. and Fulton, R. W. 1975. Understanding generates possibilities. *Phytopathology* 65:221–222.
- Ding, B. 2009. The biology of viroid-host interactions. *Annu. Rev. Phytopathol.* 47:105–131.
- Ding, S.-W. and Voinnet, O. 2007. Antiviral immunity directed by small RNAs. *Cell* 130:413–426.
- Di Serio, F., Martínez de Alba, A.-E., Navarro, B., Gisel, A. and Flores, R. 2010. RNA-dependent RNA polymerase 6 delays accumulation and precludes meristem invasion of a viroid that replicates in the nucleus. *J. Virol.* 84:2477–2489.
- Dodds, J. A. 1982. Cross-protection and interference between electrophoretically distinct strains of cucumber mosaic virus in tomato. *Virology* 118:235–240.
- Eamens, A., Wang, M.-B., Smith, N. A. and Waterhouse, P. W. 2008. RNA silencing in plants: yesterday, today and tomorrow. *Plant Physiol.* 147:456–468.
- Edwards, M. C. 1995. Mapping of the seed transmission determinants of barley stripe mosaic virus. *Mol. Plant-Microbe Interact.* 8:906–915.
- Ferguson, A. and Matthews, R. E. F. 1993. Mosaic disease induced by turnip yellow mosaic tymovirus. *Biochemie* 75:555–559.
- Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E. and Mello, C. C. 1998. Potent and specific interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391:806–811.
- Flores, R., Hernández, C., Martínez de Alba, A. E., Daròs, J.-A. and Di Serio F. 2005. Viroids and viroid-host interactions. *Annu. Rev. Phytopathol.* 43:117–139.
- Foster, T. M., Lough, T. J., Emerson, S. J., Lee, R. H., Bowman, J. L., Forster, R. L. S. and Lucas, W. J. 2002. A surveillance system regulates selective entry of RNA into the shoot apex. *Plant Cell* 14:1497–1508.
- Fraenkel-Conrat, H. 1983. RNA-dependent RNA polymerases of plants. *Proc. Natl. Acad. Sci. U.S.A.* 80:422–424.
- Fukuzawa, N., Itchoda, N., Ishihara, T., Goto, K., Masuta, C. and Matsumura, T. 2010. Hc-Pro, a potyvirus RNA silencing suppressor, cancels cycling of *Cucumber mosaic virus* in *Nicotiana benthamiana* plants. *Virus Genes* 40:440–446.
- Fulton, R. W. 1951. Superinfection by strains of tobacco mosaic virus. *Phytopathology* 41:579–592.
- Fulton, R. W. 1986. Practices and precautions in the use of cross protection for plant virus disease control. *Annu. Rev. Phytopathol.* 24:67–81.
- Gal-On, A. and Shibolet, Y. M. 2006. Cross-Protection. In “Natural Resistance Mechanisms of Plants to Viruses”. (G. Loebenstein and J.P. Carr, eds.), Springer, The Netherlands, pp. 261–288.
- Gal-On, A., Kaplan, I. and Palukaitis, P. 1995. Differential effects of satellite RNA on the accumulation of cucumber mosaic virus RNAs and the encoded proteins in tobacco vs. zucchini squash with two strains of CMV helper virus. *Virology* 208:58–66.
- García-Cano, E., Resende, R. O., Fernández-Muñoz, R. and Moriones, E. 2006. Synergistic interactions between *Tomato chlorosis virus* and *Tomato spotted wilt virus* results in breakdown of resistance in tomato. *Phytopathology* 96:1263–1269.
- García-Ruiz, H., Takeda, A., Chapman, E. J., Sullivan, C. M.,

- Fahlgren, N., Brempelis, K. J. and Carrington, J. C. 2010. *Arabidopsis* RNA-dependent RNA polymerases and Dicer-like proteins in antiviral defense and small interfering RNA biogenesis during *Turnip mosaic virus* infection. *Plant Cell* 22:481–496.
- Gilliland, A., Singh, D. P., Hayward, J. M., Moore, C. A., Murphy, A. M., York, C. J., Slator, J. and Carr, J. P. 2003. Genetic modification of alternative respiration has differential effects on antimycin A-induced versus salicylic acid-induced resistance to *Tobacco mosaic virus*. *Plant Physiol.* 132:1518–1528.
- González-Jara, P., Tenllado, F., Martínez-García, B., Atencio, F. A., Barajas, D., Vargas, M., Díaz-Ruiz, J and Díaz-Ruiz, J. R. 2004. Host-dependent differences during synergistic infection by potyviruses with PVX. *Mol. Plant Pathol.* 5:29–35.
- Goodman, R. M. and Ross, A. F. 1974a. Enhancement by potato virus Y of potato virus X synthesis in doubly infected tobacco depends on the timing of invasion by the viruses. *Virology* 58:263–271.
- Goodman, R. M. and Ross, A. F. 1974b. Enhancement of potato virus X synthesis in doubly infected tobacco occurs in double infected cells. *Virology* 58:16–24.
- Goodwin, J., Chapman, K., Swaney, S., Parks, T. D., Wernsman, E. A. and Dougherty, W. G. 1996. Genetic and biochemical dissection of transgenic RNA-mediated virus resistance. *Plant Cell* 8:95–105.
- Gottula, J. and Fuchs, M. 2009. Towards a quarter century of pathogen-derived resistance and practical approaches to plant disease control. *Adv. Virus Res.* 75:161–183.
- Hamilton, A. J. and Baulcombe, D. C. 1999. A species of small antisense RNA in post-transcriptional gene silencing in plants. *Science* 286:950–952.
- Harrison, B. D. 1958. Relationship between beet ringspot, potato bouquet and tomato black ring viruses. *J. Gen. Microbiol.* 18:450–460.
- Holmes, F. O. 1928. Accuracy in quantitative work with tobacco mosaic virus. *Bot. Gazette* 86:66–81.
- Johansen, I. E., Dougherty, W. G., Keller, K. E., Wang, D. and Hampton, R. O. 1996. Multiple determinants affect seed transmission of pea seedborne mosaic virus in *Pisum sativum*. *J. Gen. Virol.* 77:3149–3154.
- Jorgensen, R. A., Cluster, P. D., English, J., Que, Q. and Napoli, C. A. 1996. Chalcone synthase cosuppression phenotypes in petunia flowers: comparison of sense vs. antisense constructs and single copy vs. complex T-DNA sequences. *Plant Mol. Biol.* 31:957–973.
- Kasschau, K. D. and Carrington, J. C. 1998. A counterdefensive strategy of plant viruses: suppression of post-transcriptional gene silencing. *Cell* 95: 461–470.
- Kreuze, J. F., Savenkov, E. I., Cuellar, W., Li, X. and Valkonen, J. P. T. 2005. Viral class 1 RNase III involved in suppression of RNA silencing. *J. Virol.* 79:7227–7238.
- Kurihara, Y. and Watanabe, Y. 2003. Cross-protection in *Arabidopsis* against crucifer tobamovirus Cg by an attenuated strain of the virus. *Mol. Plant Pathol.* 4:259–269.
- Lakatos, L., Csorba, T., Pantaleo, V., Chapman, E. J., Carrington, J. C., Liu, Y.-P., Dolja, V. V., Fernández Calvino, L., López-Moya, J. J. and Burguán, J. 2006. Small RNA binding is a common strategy to suppress RNA silencing by several viral suppressors. *EMBO J.* 25:2768–2780.
- Lewsey, M., Palukaitis, P. and Carr, J. P. 2009. Plant-virus interactions: Defence and counter-defence. In “Annual Plant Reviews, Volume 34, Molecular Aspects of Plant Disease Resistance.” (J. Parker, ed.), Wiley-Blackwell, Oxford, pp. 134–176.
- Li, F. and Ding, S.-W. 2006. Virus counterdefense: diverse strategies for evading the RNA-silencing immunity. *Annu. Rev. Microbiol.* 60:503–531.
- Lindbo, J. A., Silva-Rosales, L., Proebsting, W. M. and Dougherty, W. G. 1993. Induction of a highly specific antiviral state in transgenic plants: implications for regulation of gene expression and virus resistance. *Plant Cell* 5:1749–1759.
- Lippman, Z. and Martienssen, R. 2004. The role of RNA interference in heterochromatin silencing. *Nature* 431:364–370.
- Lister, R. M. and Murrant, A. F. 1967. Seed transmission of nematode-borne viruses. *Ann. Appl. Biol.* 59:49–62.
- Loebenstein, G., Cohen, J., Shabtai, S., Coutts, R. H. A. and Wood, K. R. 1977. Distribution of cucumber mosaic virus in systemically infected tobacco leaves. *Virology* 81:117–125.
- Lu, R., Folimonov, A., Shintaku, M., Li, W.-X., Falk, B. W., Dawson, W. O. and Ding, S.-W. 2004. Three distinct suppressors of RNA silencing encoded by a 20-kb viral RNA genome. *Proc. Natl. Acad. Sci. U.S.A.* 101:15742–15747.
- Maule, A. J. and Wang, D. 1996. Seed transmission of plant viruses: a lesson in biological complexity. *Trends Microbiol.* 4:153–158.
- McKinney, H. H. 1929. Mosaic diseases in the canary Islands, West Africa and Gibraltar. *J. Agric. Res.* 39:557–578.
- Mérai, Z., Kerényi, Z., Kertész, S., Magna, M., Lakatos, L. and Silhavny, D. 2006. Double-stranded RNA binding may be a general plant RNA viral strategy to suppress RNA silencing. *J. Virol.* 80:5747–5756.
- Mette, M. F., Aufsatz, W., van der Winden, J., Matzke, M. A. and Matzke, A. J. M. 2000. Transcriptional silencing and promoter methylation triggered by double-stranded RNA. *EMBO J.* 19:5194–5201.
- Mink, G. I. 1993. Pollen-transmitted and seed-transmitted viruses and viroids. *Ann. Rev. Phytopathol.* 31:375–402.
- Moazed, D. 2009. Small RNAs in transcriptional gene silencing and genome defence. *Nature* 457:413–420.
- Moore, C. J. and MacDiarmid, R. M. 2006. Dark Green Islands: the Phenomenon. In: “Natural Resistance Mechanisms of Plants to Viruses”. (G. Loebenstein and J.P. Carr, eds.), Springer, The Netherlands, pp. 187–209.
- Moore, C. J., Sutherland, P. W., Forster, R. L. S., Gardner, R. C. and MacDiarmid, R. M. 2001. Dark green islands in plant virus infection as the result of posttranscriptional gene silencing. *Mol. Plant-Microbe Interact.* 14:939–946.
- Mourrain, P., Beclin, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J. B., Jouette, D., Lacombe, A. M., Nikic, S., Picault, N., Remoue, K., Sanial, M., Vo, T. A. and Vaucheret, H. 2000. *Arabidopsis* *SGS2* and *SGS3* genes are required for posttranscriptional gene silencing and natural virus resistance. *Cell* 101:533–542.

- Murphy, J. F. and Kyle, M. M. 1995. Alleviation of restricted systemic spread of pepper mottle potyvirus in *Capsicum annum* cv. Avelar by coinfection with a cucumovirus. *Phytopathology* 85:561–566.
- Napoli, C., Lemieux, C. and Jorgensen, R. 1990. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes *in trans*. *Plant Cell* 2:279–289.
- Nelson, R. S., Powell-Abel, P. and Beachy, R. N. 1987. Lesions and virus accumulation in inoculated transgenic tobacco plants expressing the coat protein gene of tobacco mosaic virus. *Virology* 158:126–132.
- Niblett, C. L., Dickson, E., Fernow, K. H., Horst, R. K. and Zaitlin, M. 1978. Cross protection among four viroids. *Virology* 91:198–203.
- Qu, F., Ye, X., Hou, G., Sato, S., Clemente, T. E. and Morris, T. J. 2005. RDR6 has a broad-spectrum but temperature dependent antiviral defense role in *Nicotiana benthamiana*. *J. Virol.* 79:15209–15217.
- Palukaitis, P. and Kaplan, I. B. 1997. Synergy of virus accumulation and pathology in transgenic plants expressing viral sequences. In: “Virus-resistant Transgenic Plants: Potential Ecological Impact.” (M. Tepfer and E. Balázs, eds.), Springer, Berlin. pp. 77–84.
- Palukaitis, P. and MacFarlane, S. 2006. Viral Counter-Defense Molecules. In “Natural Resistance Mechanisms of Plants to Viruses”. (G. Loebenstein and J.P. Carr, eds.), Springer, The Netherlands, pp. 165–185.
- Palukaitis, P. and Zaitlin, M. 1984. A model to explain the “cross protection” phenomenon shown by plant viruses and viroids. In: “Plant-Microbe Interactions - Molecular and Genetic Perspectives”. (E.W. Nester and T. Kosuge, eds.), MacMillan, New York. Volume 1. pp. 420–429.
- Pandey, S. P. and Baldwin, I. T. 2007. RNA-directed RNA polymerase 1 (RdR1) mediates the resistance of *Nicotiana attenuata* to herbivore attack in nature. *Plant J.* 50:40–53.
- Pandey, S. P., Priyanka, P., Gase, K. and Baldwin, I. T. 2008. Herbivory-induced changes in the small-RNA transcriptome and phytohormone signaling in *Nicotiana attenuata*. *Proc. Natl. Acad. Sci. U.S.A.* 105:4559–4564.
- Poolpol, P. and Inouye, T. 1986. Enhancement of cucumber mosaic virus multiplication by zucchini yellow mosaic virus in doubly infected cucumber plants. *Ann. Phytopath. Soc. Japan* 52:22–30.
- Powell-Abel, P., Nelson, R. S., De, B., Hoffmann, N., Rogers, S. G., Fraley, R. T. and Beachy, R. N. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* 232:738–743.
- Powell-Abel, P., Sanders, P. R., Tumer, N., Fraley, R. T. and Beachy, R. N. 1990. Protection against tobacco mosaic virus infection in transgenic plants requires accumulation of coat protein rather than coat protein RNA sequences. *Virology* 175:124–130.
- Pruss, G., Ge, X., Shi, X. M., Carrington, J. C. and Vance, V. B. 1997. Plant viral synergism: the potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. *Plant Cell* 9:859–868.
- Rashid, U. J., Hoffmann, J., Brutschy, B., Piehler, J. and Chen, J. C-H. 2008. Multiple targets for suppression of RNA interference by *Tomato aspermy virus* protein 2B. *Biochemistry* 47:12655–12657.
- Ratcliff, F., Harrison, B. D. and Baulcombe, D. C. 1997. A similarity between viral defense and gene silencing in plants. *Science* 276:1558–1560.
- Ratcliff, F. G., MacFarlane, S. A. and Baulcombe, D. C. 1999. Gene silencing without DNA: RNA-mediated cross-protection between viruses. *Plant Cell* 11:1207–1215.
- Rochow, W. F. and Ross, A. F. 1955. Virus multiplication in plants doubly infected by potato viruses X and Y. *Virology* 1:10–27.
- Ross, A. F. 1941. The concentration of alfalfa-mosaic virus in tobacco plants at different periods of time after inoculation. *Phytopathology* 31:410–421.
- Ruiz, M. T., Voinnet, O. and Balucombe, D. C. 1998. Initiation and maintenance of virus-induced gene silencing. *Plant Cell* 10:937–946.
- Sáenz, P., Salvador, B., Simón-Mateo, B., Kasschau, K. D., Carrington, J. C. and García, J. A. 2002. Host-specific involvement of the HC protein in the long-distance movement of potyviruses. *J. Virol.* 76:1922–1931.
- Salaman, R. N. 1933. Protective inoculation against a plant virus. *Nature* 131:468.
- Schiebel W., Pélissier T., Riedel L., Thalmeir S., Schiebel R., Kempe D., Lottspeich F., Sängler H. L. and Wassenegger M. 1998. Isolation of an RNA-directed RNA polymerase-specific cDNA clone from tomato. *Plant Cell* 10:2087–2101.
- Schmardon, A., Spoerke, J. M., Stacey, S. C., Klein, M. E., Mackin, N. and Maine, E. M. 2000. EGO-1 is related to RDNA-directed RNA polymerase and functions in germ-line development and RNA interference in *C. elegans*. *Curr. Biol.* 10:169–178.
- Schwach, F., Vaistij, F. E., Jones, L. and Baulcombe, D. C. 2005. 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.* 138:1842–1852.
- Shams-Bakhsh, M., Canto, T. and Palukaitis, P. 2007. Enhanced resistance and neutralization of defense responses by suppressors of RNA silencing. *Virus Res.* 130:103–109.
- Sherwood, J. L. 1987. Demonstration of the specific involvement of coat protein in tobacco mosaic virus (TMV) cross protection using a TMV coat protein mutant. *J. Phytopathol.* 118:358–362.
- Sherwood, J. L. and Fulton, R. W. 1982. The specific involvement of coat protein in *Tobacco mosaic virus* cross protection. *Virology* 119:150–158.
- Shi, X. M., Miller, H., Verchot, J., Carrington, J. C. and Vance, V. B. 1997. Mutations in the region encoding the central domain of helper component-proteinase (HC-Pro) eliminate potato virus X/potyviral synergism. *Virology* 231:35–42.
- Smith, J. H. 1928. The transmission of potato mosaic to tomato. *Ann. Appl. Biol.* 15:517–528.
- Smith, C. J. S., Watson, C. F., Morris, P. C., Bird, C. R., Seymour,

- G. B., Gray, J. E., Arnold, C., Tucker, G. A., Schuch, W., Harding, S. and Grierson, D. 1990. Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. *Plant Mol. Biol.* 14:369–379.
- Smith, H. A., Swaney, S. L., Parks, T. D., Wernsman, E. A. and Dougherty, W. G. 1994. Transgenic plant virus resistance mediated by untranslatable sense RNAs: expression, regulation, and fate of nonessential RNAs. *Plant Cell* 6:1441–1453.
- Sunpapao, A., Nakai, T., Dong, F., Mochizuki, T. and Ohki, S. T. 2009. The 2b protein of cucumber mosaic virus is essential for viral infection of the shoot apical meristem and for efficient invasion of leaf primordia in infected tobacco plants. *J. Gen. Virol.* 90:3015–3021.
- Vance, V. B. 1991. Replication of potato virus X RNA is altered in coinfections with potato virus Y. *Virology* 182:486–494.
- Vance, V. B., Berger, P. H., Carrington, J. C., Hunt, A. G. and Shi, X. M. 1995. 5 proximal potyviral sequences mediate potato virus X/potyviral synergistic disease in transgenic tobacco. *Virology* 206:583–590.
- van der Krol, A. R., Mur, L. A., Beld, M., Mol, J. N. M. and Stuitje, A. R. 1990. Flavonoid genes in petunia: addition of a limited number of gene copies may lead to suppression of gene expression. *Plant Cell* 2:291–299.
- van Rij, R. P. and Andino, R. 2006. The silent treatment: RNAi as a defense against virus infection in mammals. *Trends Biotechnol.* 24:186–193.
- Vanterpool, T. C. 1926. Streak or winter blight of tomato in Quebec. *Phytopathology* 16:311–331.
- Vaucheret, H. 2006. Post-transcriptional small RNA pathways in plants: mechanisms and regulations. *Genes Dev.* 20:759–771.
- Voinnet, O. 2005a. Induction and suppression of RNA silencing: insights from viral infections. *Nature Rev. Genet.* 6:206–221.
- Voinnet, O. 2005b. Non-cell autonomous RNA silencing. *FEBS Lett.* 579:5858–5871.
- Wang, D., MacFarlane, S. A. and Maule, A. J. 1997. Viral determinants of pea early browning virus seed transmission in pea. *Virology* 234:112–117.
- Wang, Y., Gaba, V., Yang, J., Palukaitis, P. and Gal-On, A. 2002. Characterization of synergy between *Cucumber mosaic virus* and potyviruses in cucurbit hosts. *Phytopathology* 92:51–58.
- Wang, Y., Lee, K. C., Gaba, V., Wong, S. M., Palukaitis, P. and Gal-On, A. 2004. Breakage of resistance to *Cucumber mosaic virus* by co-infection with *Zucchini yellow mosaic virus*: enhancement of CMV accumulation independent of symptom expression. *Arch. Virol.* 149:379–396.
- Wang, X.-B., Wu, Q., Ito, T., Cillo, F., Li, W.-X., Chen, X., Yu, J.-L. and Ding, S.-W. 2010. RNAi-mediated viral immunity requires amplification of virus-derived siRNAs in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 107:484–489.
- Wassenegger, M. 2005. The role of RNAi machinery in heterochromatin formation. *Cell* 122:13–16.
- Wassenegger, M. and Krczal, G. 2006. Nomenclature and functions of RNA-directed RNA polymerases. *Trends Plant Sci.* 11:142–151.
- Webb, R. E., Larson, R. H. and Walker, J. C. 1952. Relationships of potato leaf roll virus strains. *Res. Bull. Agric. Ex. Stat. Coll. Agric. Univ. Wisconsin.* 178:1–38.
- Wingard, S. A. 1928. Hosts and symptoms of ring spot, a virus disease of plants. *J. Agric. Res.* 37:127–153.
- Xie, Z., Fan, B., Chen, C. and Chen, Z. 2001. An important role of an inducible RNA-dependent RNA polymerase in plant antiviral defense. *Proc. Natl. Acad. Sci. U.S.A.* 98:6516–21.
- Yang, S. J., Carter, S. A., Cole, A. B., Cheng, N. H. and Nelson, R. S. 2004. A natural variant of a host RNA-dependent RNA polymerase is associated with increased susceptibility to viruses by *Nicotiana benthamiana*. *Proc. Natl. Acad. Sci. U.S.A.* 101:6297–302.
- Yelina, N. E., Savenkov, E. I., Solovyev, A. G., Morozov, S. Y. and Valkonen, J. P. T. 2002. Long-distance movement, virulence, and RNA silencing suppression controlled by a single protein in hordei- and potyviruses: complementary functions between virus families. *J. Virol.* 76:12981–12991.
- Yu, D., Fan, B., MacFarlane, S. A. and Chen, Z. 2003. Analysis of the involvement of an inducible *Arabidopsis* RNA-dependent RNA polymerase in antiviral defense. *Mol. Plant-Microbe Interact.* 16:206–216.
- Zhang, X., Du, P., Lu, L., Xiao, Q., Wang, W., Cao, X., Ren, B., Wei, C. and Li, Y. 2008. Contrasting effects of HC-Pro and 2b viral suppressors from Sugarcane mosaic virus and Tomato aspermy cucumovirus on the accumulation of siRNAs. *Virology* 374:351–360.
- Ziebell, H. and Carr, J. P. 2009. Effects of Dicer-like endonucleases 2 and 4 on infection of *Arabidopsis thaliana* by cucumber mosaic virus and a mutant virus lacking the 2b counter-defence protein gene. *J. Gen. Virol.* 90:2288–2292.
- Ziebell, H. and Carr, J. P. 2010. Cross-protection: a century of mystery. *Adv. Virus Res.* 76:211–264.