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TOBACCO MOSAIC VIRUS: A Model System for Plant Biology

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■ **Abstract** *Tobacco mosaic virus* (TMV) has had an illustrious history for more than 100 years, dating to Beijerinck's description of the mosaic disease of tobacco as a *contagium vivum fluidum* and the modern usage of the word "virus." Since then, TMV has been acknowledged as a preferred didactic model and a symbolic model to illuminate the essential features that define a virus. TMV additionally emerged as a prototypic model to investigate the biology of host plants, namely tobacco. TMV also exemplifies how a model system furthers novel, and often unexpected, developments in biology and virology. Today, TMV is used as a tool to study host-pathogen interactions and cellular trafficking, and as a technology to express valuable pharmaceutical proteins in tobacco. The history of TMV illustrates how pragmatic strategies to control an economically important disease of tobacco have had unexpected and transforming effects across platforms that impinge on plant health and public health.

> *Tobacco mosaic virus: An RNA virus that causes mosaic disease in tobacco and similar effects in other plants, much used as an experimental subject; abbrev. TMV*. (8)

INTRODUCTION

During the past five years, *Tobacco mosaic virus* (TMV) has been subjected to much prodding and poking by plant virologists and historians of science. This is due to the celebration of 100 years of virology, in particular, Martinus Beijerinck's elucidation that TMV was a *contagium vivum fluidum*, or a substance that was filterable and could move through an agar medium (i.e., a virus in modern parlance). This property provided a direct comparison with bacteria, which were not filterable and remained fixed (*contagium vivum fixum*) in agar (5, 75, 98). From this, the science of virology was established and the general features of viruses (especially that they were small and infectious) were confirmed by the work of Loeffler and Frosch on *Foot and mouth disease virus* (FMDV) (6) and the amazing discovery

by the Canadian Félix d'Herelle (79) that bacteria could be lysed by small viruses, for which he coined the name bacteriophage (bacteria eater).

As the first half of the twentieth century progressed, TMV became known as the virus of choice for basic studies in plant biology, virology (including medical virology), structural biology, biochemistry, genetics, plant disease resistance and breeding. This history has recently been discussed and placed into context, especially in *The Life of a Virus: Tobacco Mosaic Virus as an Experimental Model, 1935–1965*, as well as in several reviews and anthologies of TMV (19, 20, 75). Today, TMV holds its place as a robust tool for biotechnology and teaching, as well as an experimental and didactic model for scientists and historians.

But why did TMV develop into a tool for virology and plant biology? How did it become a standard for the practice of virology and plant biology as well as an "idea" of what defined a virus? Part of understanding the success of TMV as an object of study is to look at agricultural practices and tobacco, from the mid-nineteenth century until the early twentieth century. Why did scientists and agriculturists focus on this virus? From Beijerinck's studies, and the earlier preliminary data by Mayer and Ivanowski, there had to be a reason to be looking at the agent. One or another more myriad plant viruses have practical uses, but why does TMV persist as the right tool for so many purposes?

WHY TMV?

Before arriving at the present (and even future) status of TMV it is interesting to think about how it became a tool for biology. At the most basic, the object was not to study TMV, but to obtain a fundamental understanding of the cause of the mosaic disease of tobacco that was detrimental to tobacco production. The work on TMV was pragmatic: Mosaic disease reduced the yield and quality of tobacco. The mosaic disease was apparently familiar to tobacco growers in the Netherlands even before the first report by a student from Wageningen in 1857 (88). Adolph Mayer confirmed this observation in an 1886 report (58):

> In those regions of the Netherlands where the cultivation of tobacco flourishes, that is in the provinces of Gelderland and Utrecht, there has been prevailing for many years a disease of this cultivated plant, to which it seems to be very important to draw to the attention of the agricultural sciences; because the harm done by this disease is often very great and I myself know cases where it has caused the cultivation of tobacco to be given up entirely in a certain place.

In that manuscript (58), Mayer used the recent identification of bacteria as agents of disease and Koch's formal presentation of his postulates in 1884 (6) as a template for his research. Mayer's important advancement towards the development of virology was the "discovery that the juice from diseased plants obtained by grinding was a certain infectious substance for healthy plants. . . . [and] in nine cases out of ten one will be successful in making the healthy plant. . . heavily diseased" (58).

[Mayer also wrote a curious footnote regarding his negative controls: "Sap from healthy plants does not produce the disease, as I have proved experimentally—although to some it may seem superfluous to have tried this" (58).] The infected juice, by microscopic examination, did not yield "decisive results" but he continued by trying to "isolate these questionable organisms according to Koch's method" (58). He did sometimes culture bacteria, but in no instance were they infectious on healthy tobacco. Nematodes were also eliminated as the cause of the disease.

Although Mayer failed in his attempt to establish the etiology, having concluded that "the mosaic disease of tobacco is a bacterial disease" (58), his report can be considered "monumental in an entirely new field of thought and investigation. Mayer artificially transmitted for the first time a plant disease, the causal agent of which he demonstrated could not be seen or cultured" (45). This was during a time when "Pasteur was struggling. . . with a similar problem in rabies, and advanced little further in the direction of the cause of these peculiar diseases than did Mayer" (45). Ivanowski also reported on "a very wide-spread tobacco disease" (43) that matched the description published by Mayer, but he concluded that the small agent that could pass through porcelain filters was probably a toxin from bacteria. Therefore, not until Beijerinck's report in 1898 was it clear that the organism was smaller than bacteria. In other words, Beijerinck determined that the agent could be isolated from a filtrate of infected juice or by diffusion through agar, and then used it to infect healthy plants to reproduce the mosaic disease of tobacco (5, 75, 98).

TOBACCO AND AGRICULTURE IN THE UNITED STATES

In the early twentieth century, farming was the cornerstone of American life, with more than 40% of the people living or working on farms (73). Improvements in all aspects of agriculture met a pragmatic intent to strengthen the U.S. economy, which included local and international markets, transportation, mechanization, and the health, education, and welfare of its citizens. At the time, the political and philosophical center of the country revolved around agriculture. This dependence, and even pride, in the ability of the country to feed itself and to promote innovative agricultural practices was, in great part, a result of the Morrill Land-Grant College Act (1862), the Hatch Agricultural Experiment Station Act (1877), and the establishment of the United States Department of Agriculture (USDA) in 1862. The Homestead Act (1862) and the completion of the transcontinental railroad (1869) were the final leaps to ensure expansion of agriculture and new settlements across the United States.

As germ theory became accepted and the identification of fungi and bacteria as agents of plant diseases became more commonplace, plant pathology also took its place in improving crops and preventing plant disease at the end of the nineteenth century. The agricultural experiment stations and colleges transformed scientific research for crop improvement, and furthered the health and social well-being

of both farmers and city-dwellers (9, 68). Although the USDA was making a transition toward an emphasis on breeding for resistance and crop improvement, the understanding of pathogens was in its infancy. Many maladies had not been identified, the complex lifecycles had not been unraveled, and age-old remedies and practices were generally ineffective (9).

To understand why TMV became a model system, it seems logical to begin with an overview of tobacco. Tobacco has been a cash crop in the United States for almost three centuries, driving social, economic, and political development, particularly in Maryland and Virginia (42, 92), and even today in Kentucky and North Carolina. In Maryland, tobacco was "made legal tender in 1732 (at the rate of 1 penny per pound) for all debts, including customs dues and the salaries of State officers and ministers of the gospel. . . . As late as 1777 the tax levied for Baltimore County and city was fixed at 172 pounds of tobacco per poll" (92).

Tobacco was found throughout the Americas and the West Indies (Antilles archipelago). When the Spanish explorers arrived in what is now the Yucatan, Mexico, in the early sixteenth century, the local people were cultivating "the strange plant [tobacco] with much care and skill, and using it both for smoking and as snuff. The conquerors were themselves conquered by the gentle weed" (33) (Figure 1). The first detailed commentary on tobacco cultivation in New England was by Thomas Hariot in the late sixteenth century. He reported:

> There is an herbe which is sowed a part by it selfe & is called by the inhabitants Vppówoc. In the West Indies it hath divers names, according to the severall places & countries where it groweth and is used: The Spaniardes generally call it Tobacco. . . . We our selves during the time we were there used to suck it after their maner, as also since our returne, & have found mainie rare and wonderful experiments of the vertues thereof; of which the relation woulde require a voume by it selfe: the use of it by so manie of late, men & women of great calling as else, an some learned Phisitions [physicians] also, is sufficient witnes (66).

By 1600, tobacco had been introduced in Europe, India, Japan, Russia, possibly China, and the west coast of Africa (92). As Europeans settled in the Americas, tobacco cultivation was reported in Jamestown by 1612 and in 1619 as much as 20,000 pounds was sent to England from the colonies. Exports increased rapidly. In 1627 exports swelled to 500,000 pounds and almost 24 million pounds were shipped in 1664 (92). With this, *Nicotiana tabacum* had displaced *N. rustica* as the species under cultivation (Figure 1) (33, 92).

From 1894–1898, the United States exported ~280 million pounds of leaf tobacco each year, primarily to Europe, with a value of approximately $24 million (42, 92). By 1919, around 50% of the world crop was produced by U.S. farmers and tobacco had firmly established itself as a cash crop in southern states. For example, even with the intensive hands-on cultivation and cumbersome harvesting practices, ~457 million pounds of tobacco was produced in Kentucky in 1919 on 550,000 acres (83). The hypothetical value of all U.S. farm crops in 1919

Figure 1 Tobacco in North America. The original figure legend that accompanied this drawing by H.A. Allard reads: "Two species of tobacco cultivated extensively by the aborigines when the first colonists came to North America: *Nicotiana rustica* (*A*) and *N. tabacum* (*B*), showing buds, flowers, and green seed pods" (33).

was $16 billion; of this, tobacco was valued at $542.5 million, from an estimated production of ~1.4 billion pounds on 1.9 million acres of land (83). Tobacco was also an important source of revenue for the U.S. government. With ~8 pounds of tobacco consumed per capita in the United States in 1919 and 23 billion cigarettes available for domestic consumption (42), tobacco had become an entrenched part of American culture (Figure 2). Consumption of tobacco products per capita, aged 15 and over, in 1962 was almost 11 pounds (84). Today, per capita consumption is ~4 pounds, for those aged 18 and over (10).

With the dependence of many farmers on the tobacco crop, it was of immediate interest when the mosaic disease was reported in the Connecticut River Valley in 1898. In 1902, A.F. Woods of the USDA wrote a report that confirmed (but did not extend) the experiments of Mayer and Beijerinck (94). He noted that the mosaic disease of tobacco "occurs more or less throughout the tobacco areas of this country and is widespread in Europe wherever tobacco is grown" (94). The disease was not

easily managed, and in the late 1940s, TMV in the United States was "estimated to cause an annual average loss of 40,000,000 pounds of tobacco" (4), accounting for 2–3% of the crop. This was a significant economic concern; for example, in Kentucky from 1939–1959, tobacco accounted for one–third to one–half of the cash receipts from farming (3), and growers were in need of practical solutions to control TMV. By that time, TMV had established itself as a economically important virus and was well on its way to becoming a laboratory model for studies in plant biology, virology, genetics, and host-pathogen interactions.

TMV AND MODEL SYSTEMS

How do we go about identifying fundamental problems and extrapolation of the data toward making progress in agriculture? Model systems have been and continue to be a key in problem solving in all matters of science (2, 19, 48, 65). By definition, models become the driving force for "independent steps of discovery, invention, and development" (41). Although many organisms are promoted as model systems, few meet the standard established by TMV. Some well-established model organisms, such as mouse, *Drosophila*, yeast, and *Caenorhabditis elegans*, are essential for studies that further our understanding of cell biology and disease processes. Plant biologists have contributed to this effort with the generation of genomic sequences of *Arabidopsis* and rice, opening up a new era for genetics and integrative biology, as we compare animal, fungal, and plant genomes and gene functions (64). In plant pathology, several model organisms have been defined (69), including *Tobacco mosaic virus*, the focal point of this review. But what makes an organism (or pathogen) a model system? One defining aspect of a model system is pragmatic: It is useful. As an experimental system, TMV and tobacco have earned their reputation as a workhorse for many areas of biology, including plant pathology. TMV, as a model system, is the prototype for discussions of "what is a virus?" and it is also an exemplar (19) of how to study and understand (by analogy or directly) general mechanisms of host-pathogen interactions as well as macromolecular interactions in a normal cell.

From a practical viewpoint, the properties of TMV make it a good model system: It rapidly accumulates to high titers in infected plants; it is not transmitted by insects, fungi, or nematodes, but is easily transmitted by rub-inoculation; and TMV symptoms are easy to identify on infected plants (Figure 3). The virus also is stable for years—or even decades under ideal conditions. Another important consideration is the host range. TMV readily infects tobacco and other solanaceous plants and upwards of 200 other species, again making it very adaptable to laboratory, greenhouse, and field experimentation. Many of the well-recited "firsts" associated with TMV were in chemistry and biology, which have been elaborated, celebrated, and discussed in recent meetings and books (19, 20, 38, 75). Some of the breakthroughs in technology and/or concepts about viruses were possible

Figure 2 Finest specimen of tobacco ever grown in State of Kentucky. . .This hand of tobacco is the finest quality White Burley cigarette wrappers. Raised by Mr. B.F. Kelly, Garrad Co. Ky., and sold to S.H. Halley of the Fayette Tobacco Warehouse Co. for $15 (fifteen dollars) a pound. This hand which weighs five pounds is therefore worth $75 (seventy-five dollars) or three times the value of the same amount of silver. (With permission: Louis E. Nollau F Series Photographic Print Collection, University Archives and Records Program, Special Collection and Archives, University of Kentucky Library.) Circa 1930.

Figure 3 The mosaic disease of tobacco. Original figure legend reads: "Healthy and mosaic plants of *Nicotiana rustica*. *A*, Healthy plant; *B*, diseased plant of *Nicotiana rustica* produced by artificial inoculation from a diseased plant of *Nicotiana tabacum*" (1).

because TMV proved to be amenable to the rigors of the laboratory environment. Chemists found that "many strains of tobacco mosaic virus constitute particularly favorable material for analysis, owing to their relatively simple chemical nature and ease of purification" (19, 47).

TMV also shines as a scholastic/didactic model for many of the same reasons that it was popularized as an exceptional experimental system: It is easy and cheap to produce for classroom demonstrations, reliable in its physicochemical properties, genetics, phenotype on tobacco plants (e.g., local lesions on *N. tabacum* plants expressing the *N* gene), and amenable to testing and detection by commercially available kits. [Although the "black box" aspect of seemingly standard protocols, such as DNA plasmid preps, can make every venture in the classroom laboratory seem "experimental" (46).] Didactic models also rely heavily on symbolism, such as genetic maps, to clarify the essence of a system. For example, the TMV genome map is both practical and specific. A cartoon-like drawing (Figure 4) details the essence of the genetic make-up (5′-replicase-MP-CP-3′) of a virus that is biologically active in a host plant.

The hand-me-down nature of TMV and tradition of sharing also seems to have resulted in TMV being accepted as an experimental system, which can also be considered an extension of the didactic model. In the United States, the TMV strain used by James Johnson (Wisconsin) was distributed to many of the primary workers on TMV, including F.O. Holmes (Rockefeller), C.A. Knight (Berkeley), Sam Wildman (UCLA), and Wendell Stanley (Rockefeller, Berkeley). (This distribution also shows the robustness of the Land Grant Colleges in hiring scientists who had the wherewithal to develop TMV into an experimental system for biology.) Scientists actively made TMV, a well-calibrated material, into a benchmark or standard for research in the life sciences. TMV was immutably mobile (16, 51), both in the ease in which it was distributed to scientists in many countries, and in the fact that genetic analyses of TMV from worldwide collections were found to be remarkably (and surprisingly) similar. This finding was made possible by comparing the cDNA sequences of contemporary TMV (lab) strains with TMV isolated from tobacco leaves that were collected and stored as herbarium specimens upwards of 100 years ago (22, 30, 36).

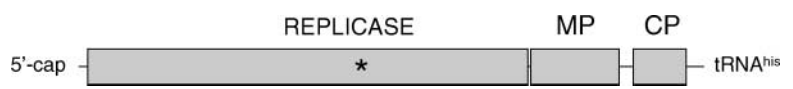

Figure 4 Genetic map of *Tobacco mosaic virus* (TMV). The ~6,400 nucleotide TMV RNA has a 5′-cap and 3′-tRNA-like structure. The open reading frames are indicated by boxes. The replicase consists of a 126-kDa protein and, by readthrough of an amber stop codon (asterisk), the 183-kDa protein. The replicase proteins are translated from the genomic RNA. The 30-kDa movement protein (MP) and the 17.5-kDa capsid protein (CP) are expressed from separate subgenomic RNAs (not shown).

A feature that thus far has set TMV apart from other model systems, such as *Drosophila*, mouse, or *C. elegans* (2, 48, 65), is that it is a "tool-box" composed of oligonucleotides, genes, and proteins that are useful to facilitate studies in basic biology and applications for biotechnology. For example, at the 5′-end of the TMV genome, a portion of the TMV leader sequence, called omega (Ω), was found to enhance translation by two- to three-fold when upstream of essentially any open reading frame on an mRNA (32), and has been incorporated in several vectors to enhance the translation of foreign genes introduced to transgenic plants. Recently, it was determined that Ω efficiently recruits eIF4F, a host factor that is required for the initiation of translation of mRNAs (31). In addition, cDNA copies of TMV subgenomic RNA promoters have been engineered into TMV-based gene vectors to engineer the stable expression of foreign genes following rub-inoculation of infectious cDNA transcripts to host plants (23, 62, 70, 74). These are but two examples of how the structure and function of a few nucleotides on the TMV RNA has both practical (toolbox) and basic research applications, extending its role as a model system.

Several TMV genes or proteins that were initially of interest for developing various types of "resistance" in plants have also become extraordinary tools, and model systems in their own right, for basic studies in plant biology. The 30-kDa MP has been used to study the mechanisms of plasmodesmatal gating and analysis of complementation of gene function [i.e., TMV MP can support movement of unrelated viruses (34)], as discussed below in detail. TMV CP cDNA was used to successfully mediate cross-protection in transgenic plants (63), and pushed the development of the plant biotechnology industry, and the subsequent (and ongoing) commentary about genetically modified organisms (72–74). This work built on the observations by H.H. McKinney that a mild strain of TMV could protect a plant from the effects by a more severe virus strain (60).

Other practical uses for TMV have been found by using the virus as a vector for the expression of foreign genes or peptides (62, 71, 74). For example, modification of the TMV genome (Figure 4) by the addition of a heterologous tobamovirus subgenomic RNA promoter to drive the transcription of the green fluorescent protein (GFP) gene allowed for direct observation of TMV movement in *N. benthamiana*, using GFP as biomolecular marker (77). Complex biochemical events in the cell that determine the fate of proteins and the induction of host responses to expression of foreign genes have also been identified by selectively silencing host proteins associated with host-pathogen resistance, as discussed below. TMV is also a good candidate to study the cascade of cellular events that occur when misfolded proteins are identified, sequestered, and/or degraded by the host, with particular attention being paid to the ubiquitin/26S proteasome pathway (44, 55, 61). These studies may be useful to model (both theoretically and experimentally) cellular and molecular interactions seemingly far removed from plant biology. For example, Parkinson's disease is associated with disruptions in the ubiquitin (Ub) pathway that interferes with normal protein degradation. TMV and tobacco might be another useful experimental model to dissect conserved complex interactions

that occur in the Ub/26S proteosome pathway (21, 44). This sort of extension of our understanding of TMV to general biology meets the confident expectations of Bawden who predicted more than 50 years ago that advances in knowledge of the behavior of viruses "will have repercussions in subjects now considered far remote from [plant] pathology" (4).

TMV AND CELL BIOLOGY

In the era of "omics" can TMV maintain its status as a model system? In a case-study format, I will show that the answer is "yes." TMV is a model system for contemporary biology, in both content and context. As we continue to decipher the molecular details of host-pathogen interactions and the biology of the eukaryotic cell, TMV will continue to shine as both exemplar and tool.

A key area where TMV been a desired tool and idea-driver is in furthering our understanding about virus movement and the determinants of the host-pathogen interaction for disease resistance. Much of this work was initiated by Francis O. Holmes (1899–1990), who made two significant observations concerning TMV that continue to reverberate in current research in plant biology and virology: (*a*) virus movement and (*b*) identification of the *N* gene for resistance (and also as a tool to quantify infections by local lesion assays) (75).

TMV Movement and Intercellular Trafficking

The most striking feature discriminating plants and animals is the basic cellular architecture. Plants have cellulose-rich cell walls that provide structural support (in lieu of a skeleton) that seemingly would prohibit intercellular communication. To overcome the ostensible limitations of a rigid cell wall, tubular connections form between the cytosol of adjacent cells, to allow for communication and transport of small molecules. These connections, or plasmodesmata, are the conduits through which viruses move in the infected plant. One of the most intriguing questions in plant biology is how TMV (as an exemplar) moves from cell-to-cell. This was addressed by production of transgenic tobacco plants expressing TMV MP. These plants provided a powerful and conclusive demonstration that the 30-kDa MP complemented cell-to-cell movement of a temperature-sensitive strain of TMV and that the functional MP localized to the plasmodesmata (PD) (24, 25). This was followed by an observation that TMV moved through the plasmodesmata as a long, thin (<2 nm width) ribonucleoprotein complex composed of the TMV genomic RNA and its MP (14, 15). The TMV MP increased the size exclusion limit (SEL) of the PD allowing the ribonucleoprotein complex to thread its way from one cell to another. This resulted in a hypothesis (a testable model) that a noninfected plant might also transport certain mRNAs from cell to cell or long distance by forming ribonucleoprotein complexes with host proteins (14). These findings opened up several new areas of research in plant biology that used TMV RNA

and MP to investigate native trafficking of host proteins and host mRNA:protein complexes.

Several related studies used TMV to investigate the mechanisms by which the transport of virus and host proteins were blocked or facilitated in tobacco. TMV and tobacco were at the forefront of determining how virus movement proteins caused physical changes to the PD that allowed for transit of large macromolecules. A decade ago, a rush of studies showed that MP alone, either expressed as a transgene or by microinjection into cells, allowed for direct measurements of the size exclusion limits using fluorescently labeled sized-dextran markers. That the MP accumulated at the PD led to other observations related to plant cell development. For example, TMV MP is biologically active when it is phosphorylated, which pointed to the finding that kinases are associated with the PD. In turn, this suggested that the host used the PD as a gating mechanism to regulate protein transit from cell-to-cell (13, 67, 85). Furthermore, host proteins also can contribute to or preclude movement through PD. Pectin methylesterase (PME) is needed for normal growth and development of the plant cell (12, 27) and it also may be involved in TMV spread. TMV movement-defective mutants bind PME less efficiently and cell-to-cell movement is blocked. In another experiment, gene silencing was used to reduce PME expression by ~80% in the plant cell, delaying the initiation of long-distance (systemic) spread. Subsequent unloading of TMV to nonvascular tissue, one aspect of systemic infection, was also inefficient (11). This suggested that movement is a polar process, or regulated, in that ingress of macromolecular complexes (such as TMV-MP) to the vasculature did not guarantee subsequent egress to nonvascular tissues (11, 35). These findings will likely have practical applications in using the plant to interfere with virus infections as well as to look more deeply into normal vascular transport of macromolecules. Within the above context, host proteins and cellular architecture facilitate TMV replication and movement. For example, TMV replication occurs on membranes associated with the endoplasmic reticulum (ER) (57).

Other plant macromolecular complexes have been defined as essential components of host defense and the ability of TMV to exploit such complexes for movement. For example, TMV replication has been identified as occurring on membranes associated with the endoplasmic reticulum (ER) (57). In addition, MP is colocalized at the ER. How does the TMV RNA:MP complex get to the PD? Is TMV MP able to traffic/localize to the PD and increase the SEL by associating with virus RNA? What is the role of microtubules in this association? Several of these aspects have been clarified, including that there apparently are at least two routing strategies for the MP. Elegant studies with a TMV MP mutant showed that its ability to move to the PD and increase the SEL can be separated from its targeting to microtubules. From this, it was determined that at least a portion of the TMV MP is regularly scavenged by a microtubule-associated protein (MPB2C) from *N. tabacum*, as a possible means to interfere with (or compete with) cell-to-cell transport (49). Thus far, the cellular role of this constitutively expressed ~36-kDa protein is unknown. However, it is quite possible that localization to microtubules may trigger ubiquitinylation and degradation, a host housekeeping response to dispose of misfolded or aggregated proteins. Recent studies suggest

that an interaction between the TMV 126-kDa protein and the *N*-gene protein triggers degradation thorough a Ub/26S proteasome pathway, as discussed below. Again, TMV and its encoded proteins are excellent candidates towards modeling both housekeeping and defense strategies in the plant cell.

Other *N. tabacum*-encoded proteins have been identified as having an intimate role in guiding MP to the PD, including NCAPP1, a 40-kDa protein with an amino-terminal transmembrane domain that targets the protein to the ER. The role of this protein was defined in part by a 22-amino-acid deletion mutant (NCAPP$\Delta_{1–22}$) that hampered the ability of both the TMV MP and an endogenous RNA-binding host-protein (CmPP16) to increase the plasmodesmal SEL (52, 96). It appears that the NCAPP1 protein (alone or as a component of a macromolecular complex or pathway) is associated with guiding the MP to the PD and/or translocating it from cell to cell. Interestingly, silencing NCAPP1 or overexpression of NCAPP$\Delta_{1–22}$ (as a dominant-negative mutant) resulted in tobacco with altered developmental phenotypes including thickened and misshaped leaves, infertile flowers, and lack of organ symmetry (52).

The determination that TMV MP traffics RNA as a ribonucleoprotein complex resulted in many advances in our understanding of the regulation of normal host cell development (39, 82). For example, KNOTTED1, a maize homeobox protein, increased the PD SEL and specifically moves its own mRNA (56, 82). Of equal importance is that these studies have led to recent findings that some proteins, such as LEAFY, another transcription factor, move in a nontargeted fashion (akin to diffusion) unless there is a specific block (95). This transitions into intriguing questions about the mechanisms by which the apical meristem generally excludes nascent virus infections. Each of these studies have shown that TMV RNA and MP can be used both as a tool to stand-in for normal host function and to probe for events that are associated with normal intra- and intercellular trafficking and development.

Host Resistance to TMV

N-gene-mediated resistance to TMV is at the forefront of recent advances in defining the molecular basis of host response to disease. The impetus for this work was based on practical studies by F.O. Holmes, one of the true pioneers in plant virology (76). Holmes, based on earlier reports by Allard (1), developed a stable hybrid of *N. tabacum* × *N. glutinosa* to contain infections of TMV to necrotic primary lesions (40). He described this gene from *N. glutinosa* "as *N* (necrotic-type response to infection with tobacco-mosaic virus)" (40). Holmes reported that tobacco lines with the necrotic-type response protected the plants "by early death of invaded tissues, with consequent imprisonment of most of the virus," that the *N* gene was dominant, and that a quick bioassay for the *N* gene could be made by rub-inoculation (40), i.e., a local lesion assay. The isolation and molecular characterization of the *N* gene resulted in yet another successful transition by TMV from the field to lab (and possibly back to the field again), with the demonstration that the expression of the *N* gene in transgenic tomato conferred protection against TMV infection (89, 90).

From solving a practical problem of putting disease resistance in the field (the *N* gene), TMV has led us to a fascinating picture of the complex biochemical interactions that occur in normal and virus-infected cells. On the host side, structure-function analyses of the TMV N protein have clarified some aspects of the mechanism of the R-gene response to TMV infection (89, 90). Under ideal conditions, *N*-gene plants that are infected with TMV are protected from systemic infection by two events: induction of the HR, resulting in localized necrosis (cell-death), and a separate cascade of events that results in systemic acquired resistance (97). For this discussion the focus is on the localized cellular events and dissection of host-encoded complexes that are associated with the TMV infection and how TMV and tobacco are being used as a tool and a model system for such studies.

The N protein has several features now known to be essential for activation of innate immunity following "detection" of an avirulence factor (29), in this case the TMV 126-kDa replicase-associated protein required for TMV replication. The N protein has three signature motifs common to many other plant R genes (29). This class of R genes (TIR-NBD-LRR) is defined by a Toll/interleukin-1 receptor-like domain (TIR), a nucleotide-binding site (NBD), and a leucine-rich repeat (LRR) (26). The LRR domain is implicated in interacting with the C-terminal half of the 126-kDa TMV replicase protein that is defined as an avirulence determinant (26, 53).

The biochemical interactions between the host cell machinery and TMV are being dissected by direct approaches based on yeast two-hybrid assays to identify host proteins that interact with the TMV replicase (in particular the 50-kDa region on the C-terminal portion of 126-kDa replicase protein; Figure 4). This strategy has resulted in the identification of several tobacco proteins that are associated with responses to cellular stresses, including heat shock proteins (Hsp) and components of the Ub/26S proteasome pathway (53, 54, 61). Gene silencing has been used to confirm some of the interactions, and in each case, it has reduced or abolished the *N*-gene response to TMV infection. For example, when Hsp90 was suppressed, it interfered with both plant growth and development and *N* gene-mediated resistance (53). Gene arrays also hold promise for the identification of host mRNAs that are up- or down-regulated in response to virus infection (37, 91). This should broaden the scope of investigations and will likely show some convergence of the sentinel features associated with the success of TMV in using host factors as well as the cascade of events that occur in the cell in response to TMV and other virus infections (91).

Even classic events such as cross protection are open for molecular reassessment. For example, an attenuated strain of *Tomato mosaic virus* (ToMV L_{11}A) has been used for at least 20 years to protect commercial lines of tomato (87). ToMV L_{11}A was identified as a good cross-protecting strain because it produces no symptoms and moves systemically. This protective effect was narrowed down to a single amino acid change in the 126-kDa region of the TMV replicase gene. The recent determination that this attenuated strain is permissive for posttranscriptional gene silencing provides a mechanism by which L_{11}A protects host plants from infection

by severe strains of ToMV (50). This also is a powerful demonstration that our understanding of even "simple" practical uses of TMV for pathogen-derived resistance will undergo further evaluation and study. TMV, not only being the right tool for many jobs, continues to open up new avenues for understanding basic molecular features common to eukaryotic cells.

TMV: TOBACCO AND HEALTH?

Any discussion of tobacco and TMV is complicated by social, policy, health, legal, agricultural, and economic issues that bring forth strong opinions and emotions related to individuals rights and supporting family (versus corporate) farmers (86). In 1964, the Surgeon General's Report, representing more than 50 years of data, clearly stated the detrimental effects of cigarette smoking on health (84). The Centers for Disease Control and Protection (CDC) reported that in the United States from 1995–1999 there were 440,000 smoking-related deaths each year, costing $150 billion in health-related economic losses. The World Health Organization estimates that there are 4.9 million tobacco-related deaths per year and predicts a doubling of that number by 2030. In 1998, a Master Settlement Agreement between tobacco companies and states' attorneys general resulted in a payout of up to $246 billion by 2025 that is intended for antismoking education and related public health programs. Antismoking campaigns and legislation have resulted in smoke-free public areas and a reduction of quotas for tobacco production. Tobacco price supports/quotas were established by the U.S. government in the 1930s with the intension of restricting supply to guarantee a minimum price at market (78). Quotas have declined and a final buyout is likely. Bills are pending in both houses of the U.S. Congress (108th Legislative Session) to eliminate the quota program and to compensate growers and absentee quota owners for lost value (78, 93).

In 2003, US tobacco production was forecast at 844 million pounds grown on 413,010 acres, which would be the smallest crop since 1908. Tobacco yields for 2003 are expected to average 2044 pounds per acre, with estimated value of $1.89 per pound. With ~100,000 cigarettes produced per acre, the tax revenue is still substantial with an average state excise tax of $0.59 per pack of cigarettes. Combined, more than $50,000 in federal, state, and local taxes are generated from an acre of tobacco (78). Today, Kentucky has more than 50% of all tobacco farms in the United States (7) and the state economy is only now addressing a need to reduce its dependence on tobacco. In Kentucky, in 2000, tobacco accounted for $900 million, or 36% of the net cash receipts from agricultural sales, surpassed only by horses and cattle (78). Historically, tobacco has been regarded as a "Christmas crop," providing supplemental income to small farmers; 45% of Kentucky tobacco farms have tobacco sales income of less than $10,000 (78).

So, what practical value is there for TMV and tobacco in the near future? Tobacco, a high biomass plant, is historically a valued drug (and sometimes medicine) (18) and a known agricultural poison—nicotine being sought after as the active

ingredient in each case. At its most base, it is "the evil weed" and regulatory efforts to reduce the incidence of smoking (by taxation and legislation) should be applauded. In any case, it is clear that within the coming decade, price supports and the economic impetus for continuing tobacco production will come to an end.

However ironic, tobacco, tobacco farmers, and rural communities may enjoy a healthy future in the United States with the recent demonstrations that tobacco is a good host plant for biotechnology and pharming. Using reverse genetics, the cDNA of TMV and other plant viruses have been engineered into useful vectors to "carry" foreign genes into plant cells (70, 71, 74). One demonstration of the promise of TMV for agriculture is the production of a new tobacco species, *N. excelsiana* (28, 62), a cross between *N. excelsior* and *N. benthamiana*; both plants are indigenous to Australia. This plant is of short stature, with a bushy habit, and resistance to blue mold (*Peronospora tabacina*) fungal infections. *N. excelsiana* tends to produce lateral buds and this new growth allows TMV to continually move into new tissues, thus producing higher levels of valued-added product (G. Pogue, personal communication). The plant also matures faster than *N. tabacum*, allowing for the plants to be harvested within 4 weeks of being placed in the field.

Recent improvements to TMV infectious cDNA clones have increased the stability and expression of foreign gene inserts (62, 80). By making either TMV-CP-fusions or using the virus vector to overexpress free protein products, Large Scale Biology Corp. (LSBC; Vacaville, CA) has been able to produce as much as 500 mg/kg of high-value proteins or peptides in *N. excelsiana*. The retail value of many of the recombinant proteins fall in the range of $1000-$100,000/g. Plant-derived recombinant proteins may reduce wholesale costs of producing the expensive active ingredients that are used to formulate innovative medicines that improve our quality of life or are life-saving. Using the TMV-expression system, LSBC has developed extraction and purification methods to isolate recombinant proteins from tobacco that have been used in, and safely administered to, humans in U.S. Food and Drug Administration approved clinical trials (59, 62, 81). With an aging population, escalating prescription drug costs, and reduced access to medicines [an estimated 41 million Americans are without health insurance (17)], it is imperative that we explore new ideas and strategies that may reduce retail drug costs and benefit public health. Pharming may provide a practical method to lessen the enormous costs typically associated with bulk production of high-value proteins and in turn, we can hope this will result in a concomitant reduction in the price of retail prescription medicines.

What is the role of the traditional tobacco grower in this? Interestingly, the farmers are the critical link in the production side of this process. The innovative tobacco farmer will benefit the most from this transition, providing the expertise to produce high-quality plants in the field and to ensure that farming remains a viable option, using a high-value crop, generally in rotation with maize and soybeans. Although only 10–15 acres of *N. excelsiana* are currently in production, the USDA

has issued a policy statement that LSBC can expand tobacco production to include 1000 acres of cultivation under appropriate consultation and approval (G. Pogue, personal communication). Therefore, there is significant opportunity to develop new (and oftentimes novel) linkages between industry, farmers, and society. In the coming decade, TMV and tobacco may transform the pharmaceutical industry and provide one more income option for small farmers in tobacco-growing states.

CONCLUSION

In the 100 years since the mosaic disease of tobacco was first described as a *contagium vivum fluidum*, TMV has made a remarkable transition—it is a valued tool for agriculture and is back in the field. Thus, TMV and tobacco have come full circle. TMV has proven itself in the past, and it is likely that it will continue to be "reinvented in response to unexpected results, new strategies, and unseen opportunities" (19). The future is bright for TMV, including new uses as a tool to improve crop resistance, especially by deploying the *N* gene to solanaceous crops, developing new strategies to block movement and/or replication by continuing studies on trafficking, and expressing value-added proteins in plants. It also retains its role as a powerful model system to dissect and direct basic studies in plant biology, with possible implications for general biology including macromolecular movement, protein (mis-)folding and degradation, and coevolutionary processes that determine innate and gene-for-gene resistance mechanisms.

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