MEETING REPORT

artifactual aggregation involving CLV1.) Their intriguing hypothesis is that the CLV3 protein may in fact be the activating ligand of the CLV1 receptor kinase. This possibility is attractive in that intermolecular associations of protein kinases are known to be instigated in animal systems by the binding of ligands to cell receptors. As the authors point out, the cloning of *CLV3* will help to elucidate the signal transduction pathway that is promoted by CLV1 to regulate meristematic function.

Harry B. Smith

REFERENCES

Clark, S.E. (1997). Organ formation at the vegetative shoot meristem. Plant Cell **9,** 1067–1076.

Clark, S.E., Williams, R.W., and Meyerowitz, E.M. (1997). The *CLAVATA1* gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis. Cell **89,** 575–585.

Hua, J., Sakai, H., Nourizadeh, S., Chen, Q.G., Bleecker, A.B., Ecker, J.R., and Meyerowitz, E.M. (1998). *EIN4* and *ERS2* are members of the putative ethylene receptor gene family in Arabidopsis. Plant Cell **10,** 1321–1332.

Lease, K., Ingham, E., and Walker, J.C. (1998). Challenges in understanding RLK function. Curr. Op. Plant Biol. **1,** 388–392.

Ridley, A.J., and Hall, A. (1992). The small GTP-binding protein Rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell **70,** 389–399.

Solano, R., and Ecker, J.R. (1998). Ethylene gas: Perception, signaling and response. Curr. Op. Plant Biol. **1,** 393–398.

Stone, J.M., Collinge, M.A., Smith, R.D., Horn, M.A., and Walker, J.C. (1994). Interaction of a protein phosphatase with an Arabidopsis serine–threonine receptor kinase. Science **266,** 793–795.

Stone, J.M., Trotochaud, A.E., Walker, J.C., and Clark, S.E. (1998). Control of meristem development by CLAVATA1 receptor kinase and kinase-associated protein phosphatase interactions. Plant Physiol. **117,** 1217–1225.

Tari, A.M., Hung, M.C., Li, K., and Lopez-Berestein, G. (1999). Growth inhibition of breast cancer cells by Grb2 downregulation is correlated with inactivation of mitogen-activated protein kinase in EGFR, but not in ErbB2, cells. Oncogene **18,** 1325–1332.

Trotochaud, A.E., Hao, T., Wu, G., Yang, Z., and Clark, S.E. (1999). The CLAVATA1 receptor-like kinase requires CLAVATA3 for its assembly into a signaling complex that includes KAPP and a Rho-related protein. Plant Cell **11,** 393–405.

Williams, R.W., Wilson, J.M., and Meyerowitz, E.M. (1997). A possible role for kinase-associated protein phosphatase in the Arabidopsis CLAVATA1 signaling pathway. Proc. Natl. Acad. Sci. USA **94,** 10467–10472.

Tobacco Mosaic Virus: Pioneering Research for a Century

One century ago, M.W. Beijerinck contended that the filterable agent of tobacco mosaic disease was neither a bacterium nor any corpuscular body, but rather that it was a *contagium vivum fluidum* (Beijerinck, 1898). Beijerinck's contribution followed A. Mayer's path-breaking work on tobacco mosaic disease and D. Ivanowski's demonstration in 1892 that the agent of tobacco mosaic disease could pass through a filter capable of retaining bacteria (Mayer, 1886; Ivanowski, 1892; Zaitlin, 1998). The claim that viruses, lacking cells, were nonetheless living, set off a scientific debate about the nature of life that animated biology for decades.

Tobacco mosaic virus (TMV), as we now know the agent that Beijerinck and others were studying, was the first virus to be identified. Perhaps because of this, research on TMV and other plant viruses has continued to be of profound significance in addressing fundamental questions about the nature of viruses in general. Indeed, TMV as a model system has been at the forefront of virology research to the present time. For example, TMV was the first virus to be chemically purified (Stanley, 1935; Bawden et al., 1936), to be detected in an analytical ultracentrifuge and in an electrophoresis apparatus (Eriksson-Quensel and Svedberg, 1936), and to be visualized in an electron microscope (Kausche et al., 1939). TMV RNA was used in the first decisive experiments showing that nucleic acids carry hereditary information and that nucleic acid alone is sufficient for viral infectivity (Fraenkel-Conrat, 1956; Gierer and Schramm, 1956). The TMV coat protein (CP) was the first virus protein to be sequenced (Anderer et al., 1960; Tsugita et al., 1960), and TMV's particle structure was among the first to be elucidated in atomic detail (Bloomer et al., 1978; Namba et al., 1989).

TMV's preeminence has extended into the recombinant era, when the first transgenic plants were constructed using TMV to demonstrate the concept of CP-mediated cross-protection (Abel et al., 1986). TMV was also the first virus shown to encode a cell-to-cell movement protein (MP; Deom et al., 1987). MP binds to RNA (Citovsky et al., 1990), associates with cytoskeletal elements (Heinlein et al., 1995; McLean et al., 1995), and increases the permeability

MEETING REPORT

of plasmodesmata to mediate cell-to-cell movement of the virus (Wolf et al., 1989; Waigmann et al., 1994).

Several properties of TMV have made it particularly amenable to laboratory investigation. For example, infected tobacco plants produce TMV so abundantly that inclusion bodies of crystallized virions in the infected leaves are visible under the light microscope. Moreover, TMV is not transmitted by insects, nematodes, or other vectors; it infects cells via direct contact with wounded areas on plant surfaces. Virus infection causes disease by preventing chloroplast development, resulting in stunted plants with leaves showing a characteristic mosaic pattern of light and dark green. Furthermore, TMV is remarkably stable: its in vitro longevity in infected sap is 3000 days, and purified virions kept at 5°C remain viable for at least 50 years. Virus stability derives directly from the densely packed structure of the viral particles, which consist of a single genomic RNA molecule enclosed in a cylindrical protein coat. TMV virions have a regular length of 300 nm and a width of 18 nm; these rods comprise a tight array of 2130 identical CP subunits, each containing 158 amino acids.

The TMV RNA genome is single stranded and linear, with a length of ~6400 bases. The complete TMV nucleotide sequence was first determined for the U1 strain (Goelet et al., 1982), and comparisons between this sequence and the RNA sequences of other TMV strains have revealed a tightly organized genome that encodes at least three nonstructural proteins (P126, P183, and the 30-kD MP), a putative 54-kD protein of unknown function, and the CP (Figure 1). Both P126 and P183 function as components of the TMV replicase (Palukaitis and Zaitlin, 1986) and are translated directly from the genomic TMV RNA; P183 is produced by read through of the amber termination codon of P126 (Pelham, 1978). In addition, there is a start codon within this read-through region and an open reading frame that could potentially encode the putative 54-kD protein. Translation of MP and CP occurs from the I_2 RNA and CP subgenomic (sg) RNAs, respectively (Figure 1). Whereas P126, P183, and CP are continuously expressed (Watanabe et al., 1984), translation of MP is transient, occurring early in the infection process (Joshi et al., 1983; Watanabe et al., 1984).

To celebrate the first century of TMV research, scientists from around the world gathered at the Royal College of Physicians of Edinburgh, Scotland on August 7 and 8, 1998, for a symposium sponsored by the Royal Society of Edinburgh in association with The Royal Society, London, UK. The meeting was organized by Professors Bryan D. Harrison and T. Michael A. Wilson (both of the Scottish Crop Research Institute, Dundee, UK) to consider how studies on TMV have contributed to the fundamental knowledge base of biology. A wide diversity of research fields—crystallography, plant pathology, immunology, biochemistry, genetics, and evolutionary biology—was represented at the meeting, with distinguished symposium speakers describing the contributions made by their respective disciplines to our current understanding of TMV biology. The overview they provided made clear that the special status of TMV as a research object has depended on its biological characteristics as well as upon its historical status as a virus of many "firsts."

In the remainder of this report, we describe symposium presentations focused on four central approaches to TMV research—structural biology, genetics and evolution, cell and molecular biology, and biotechnology—and we emphasize the impact that research on TMV has had among the life sciences over the course of the twentieth century.

Structural Biology

The nature of the three-dimensional architecture of viruses and the assembly of viral subunits and nucleic acids have been among the central issues in virology over the past fifty years. Sir Aaron Klug (Medical Research Council Laboratory, Cambridge, UK), President of the Royal Society of London, offered his own historical perspective on the resolution of TMV architecture and its implications for virus self-assembly. Klug

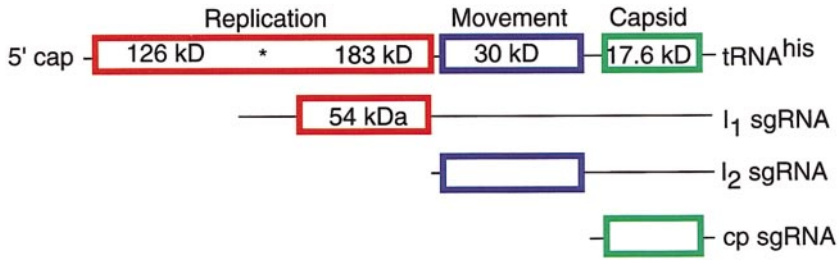

Figure 1. Current Molecular Biological Conception of TMV, as Represented by a Schematic Diagram of its Genome Organization.

The genomic RNA is shown on top and the 3′ coterminal subgenomic (sg) RNAs are shown underneath. The predicted molecular weights of proteins encoded by the open reading frames (boxes) are given in kilodaltons, and the functions of individual genes are indicated (the function of the putative 54-kD protein is unknown). The asterisk denotes the amber read-through codon.

began working with R. Franklin in 1954, just two years before the first big picture of TMV quaternary structure emerged (Franklin et al., 1956). This picture was based largely on the high-quality x-ray photographs Franklin obtained from her samples of repolymerized, nucleic acid–free TMV particles (Franklin, 1955). Franklin thus confirmed J.D. Watson's deduction that the rod-shaped virus was helical (Watson, 1954), but she also provided evidence that the helix was hollow rather than solid and that TMV RNA was embedded in the protein helix (Caspar, 1956; Franklin, 1956). Experimental evidence from these studies on TMV provided the basis for F.C. Crick and Watson's contention that all viruses must be built up symmetrically from identical protein subunits that surround the nucleic acid (Crick and Watson, 1956). The elegant simplicity of this observation prompted the witticism, attributed to Crick, that "Any child could make a virus." In listening to the participants at the Edinburgh symposium, one could not help but note that TMV research has been a serious playground (*pace* Max Delbrück) for some of the most formidable structural biologists of the twentieth century.

The intensive study of the structure of TMV has established it as one of the best-investigated models of macromolecular organization in biology. The classic reconstitution experiments, in which complete TMV was produced in vitro by mixing purified virus RNA and protein subunits (Fraenkel-Conrat and Williams, 1955), demonstrated that the information required for assembly is present in the structural components of the virus. Subsequent studies of the self-assembly of the virus have drawn from the wealth of biophysical and biochemical data on the various stable aggregates of TMV CP, as Donald L.D. Caspar (Florida State University, Tallahassee) noted in his contribution to the symposium. By the late 1950s, M. Lauffer's physicochemical studies of the TMV CP along with Caspar's own titration studies had provided evidence that disks comprising two cylindrical layers of 17 subunits each might serve as important intermediates for the assembly of virus helices (Lauffer et al., 1958; Caspar, 1960).

During the ensuing four decades, a great deal of progress has been made in resolving the structure and function of these 34-subunit disks, although the degree to which disks are involved in virus assembly remains controversial. P.J.G. (Jo) Butler (MRC Laboratory of Molecular Biology, Cambridge, UK) recounted the evidence amassed by him, Klug, and their coworkers showing that virus disks are essential for self-assembly. According to their model, nucleation is initiated by the binding of an internal sequence of TMV RNA to a disk, which then dislocates into a helical structure. Other dislocated disks associate with the initiation complex to form nicked helices. Over time, the protein subunits realign and anneal into an uninterrupted helical rod (Butler, 1984). Butler presented kinetic evidence implicating the disks in rod elongation as well as nucleation.

Butler and Klug's assertions did not go unchallenged, however. Marc H.V. Van Regenmortel (Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France) pointed out that only polar disks can form helices. The stacked disks produced by most in vitro experiments are bipolar, and thus cannot, in his view, represent intermediates in the assembly process. Recognizing that the problem of TMV self-assembly has not been entirely settled, Klug reminded the audience of A.N. Whitehead's famous dictum, "It is more important that an idea be fruitful than correct."

The desire to ascertain the structure of complete TMV, rather than only of smaller oligomeric subunits, has required the development of innovative crystallographic methods. Gerald Stubbs (Vanderbilt University, Nashville, TN) recalled a 1971 talk on TMV structure by K.C. Holmes that inspired his efforts to push the resolution of TMV structure below 10 Å. The conventional reliance on cylindrical averaging of the fiber diffraction data, although yielding the overall architecture of the virus, had effectively obscured more detailed structural information.

Stubbs and his coworkers developed new isomorphous replacement and computational techniques to achieve atomic resolution of intact TMV, providing new structural details of both the RNA and the capsid subunits, and by 1989, they had obtained a 2.9-Å map of TMV (Namba et al., 1989). Stubbs pointed out that the techniques developed to extend the resolution of TMV have been useful for investigating other complex biological structures, including filamentous bacteriophages.

Virus Genetics and Evolution

As Yoshimi Okada (Teikyo University, Utsunomiya, Japan) reminded those attending the symposium, it was not until the 1950s that most biologists accepted that genes are constructed from nucleic acids. Experiments with TMV played an important role in this development by providing the first unequivocal demonstration that a viral RNA molecule—specifically the TMV RNA—was sufficient for infectivity and carried all of the information necessary for synthesis of the CP (Fraenkel-Conrat, 1956; Gierer and Schramm, 1956).

Bea Singer (University of California, Berkeley) recounted how she and H. Fraenkel-Conrat (University of California, Berkeley) extended biochemical research of TMV genetics. They began with naturally occurring TMV strains to demonstrate that the progeny of mixed viruses (i.e., protein from one strain and RNA from another) were true-to-type for the TMV nucleic acid (Fraenkel-Conrat and Singer, 1957). Fraenkel-Conrat and Singer subsequently employed the mutagen nitrous acid (Gierer and Mundry, 1958) to generate novel

MEETING REPORT

variants of TMV that were then compared in terms of their nucleic acid content, CP composition, and disease symptoms. Due to the labile nature of the TMV RNA, these were difficult experiments. As Singer recalled, she and Fraenkel-Conrat protected the RNA from cellular RNases by adding the clay bentonite, leading their colleague C.A. Knight to remark that he "wouldn't put that mud in his stuff."

Singer asserted that her work with Fraenkel-Conrat represented the true beginning of chemistry applied to virology. However, one might well point out that this work drew on concurrent developments in bacteriology and bacterial genetics, beginning with research performed a decade earlier at the Rockefeller Institute, where Avery and his coworkers biochemically demonstrated the "transforming principle" of *Streptococcus* to be DNA.

With the elucidation of the complete CP sequence in 1960 (Anderer et al., 1960; Tsugita et al., 1960) the collection of TMV mutants provided clues used to crack the genetic code. Only the startling development of cell-free translation systems the following year by H. Matthei and M. Nirenberg provided a less laborious means to decipher this code (reviewed in Kay, 1998), and even then TMV mutants were used to confirm the emerging codon dictionary.

The TMV mutants shed light on other biological questions as well. As Singer also noted, almost all the mutants attributed to the nitrous acid treatment were less "fit" than was wild-type TMV, an observation suggestive of later developments in the arenas of virus diversity and evolution.

Milton Zaitlin (Cornell University, Ithaca, NY) recalled how advances in molecular genetic techniques enabled researchers in the 1970s and 1980s to construct a detailed map of the TMV genome. Indeed, a significant clue to the genetic composition of TMV RNA came from studies in Zaitlin's laboratory showing that a low molecular–weight component termed sgRNA accumulated during viral infection (Jackson et al., 1972). This sgRNA was soon implicated as the mRNA that directs CP production (Hunter et al., 1976). By the mid-1970s, Zaitlin's group had correctly, albeit tentatively, placed the replicase-encoding gene at the 5′ end, the CP-encoding gene at the 3′ end, and a gene necessary for viral movement in the central portion of the genome (Beachy et al., 1976; see Figure 1). Nishiguchi, Okada, and coworkers (Nishiguchi et al., 1978; Ohno et al., 1983) then confirmed that the 30-kD MP was encoded by TMV using TMV strain L and a temperature sensitive variant, Ls-1. Several reverse genetics studies using infectious clones, first assembled in 1986 (Dawson et al., 1986; Meshi et al., 1986), have confirmed the gene functions assigned during these earlier studies.

The initiation of TMV infection and disassembly of the TMV virion was reviewed by John G. Shaw (University of Kentucky, Lexington). Having entered its host cell, the TMV virion must remove its CP to enable viral replication. Shaw presented one model describing how this uncoating might take place bidirectionally, proceeding both from the 5′ and the 3′ ends of the TMV genomic RNA molecule. The 5′-to-3′ uncoating reaction may be cotranslational (Wilson, 1984), which would result in disassembly by a ribosome-mediated mechanism and concomitant protection of the uncoated viral RNA from cellular nucleases. Shaw suggested that the 3′-to-5′ uncoating reaction might occur in a coreplicational manner, because viral replicase mutants that are defective in 3′-to-5′ disassembly can be uncoated in plant protoplasts by adding free viral RNA with an intact replicase gene (Wu and Shaw, 1996, 1997). These studies of TMV infection at the molecular level exemplify the highly efficient coordination of seemingly disparate events associated with virus replication.

Ken Buck (Imperial College of Science, Technology, and Medicine, London, UK) dissected the process of TMV replication, and he noted that although the viral proteins involved in TMV replication are well characterized, the involvement of host factors is poorly understood. On the basis of their physical association with the viral replicase proteins, Buck proposed two candidate host proteins that might be involved in TMV RNA synthesis: EF-1α, which colocalizes with the replicase complex, and a subunit of eIF-3, which copurifies with the replicase. In addition, Buck mentioned genetic approaches to dissect virus–host interactions that have led to the identification of the *tom-1* and *tom-2* mutants of Arabidopsis and the *Tm-1* mutant of tomato, in which TMV replication is restricted.

Population genetic studies with tobamoviruses have also yielded surprising results. Although RNA viruses have the potential to vary more widely than DNA viruses (Domingo and Holland, 1994), TMV provides a case of high genetic stability. Adrian J. Gibbs (Australian National University, Canberra) compared cDNA sequences of recent isolates of tobacco mild green mottle tobamovirus (TMGMV) and TMV with those derived from infected *Nicotiana glauca* specimens deposited in Australian herbaria since 1899. Gibbs reported that these analyses demonstrate that there has been no increase in the genetic diversity of TMGMV in Australia over the past 100 years. Moreover, the mutations observed in TMV seem to be deleterious because TMGMV has become the more dominant tobacco virus in *N. glauca* in that country (Fraile et al., 1997).

More generally, tobamoviruses from places as far removed as California and Crete appear to be part of one large world population with very limited variation. This remarkably constrained variation suggests that, despite varying selective pressures, the viral genome remains generally immutable as a result of long-term host–virus interactions. In other words, there appears to be a re-

stricted window of TMV sequence variability, outside of which the host plant's ability to recognize and repulse this pathogen is greatly enhanced. In this respect, TMV appears to be very different from other viruses, such as influenza and HIV, which characteristically exhibit high rates of nucleotide change. The restricted variation characterizing TMV worldwide likely aided early virologists, who were able to duplicate results from distant laboratories with relative ease.

Cell and Molecular Biology

Invading pathogens have generally evolved to insinuate themselves into the metabolic pathways of their hosts and adapt these pathways for their own use. Thus, studies of virus–plant interactions are not only valuable for furthering understanding of the viral life cycle and the mechanisms of viral diseases, but they also shed light on such general cellular processes as the regulation of gene expression, hormonal responses, intercellular communication, and molecular transport. Historically, TMV has often served as the experimental system of choice for these lines of research. Indeed, as Harrison noted, F.C. Bawden and N.W. Pirie first suggested in 1936 that virus replication might be analogous to the synthesis of cellular components.

Several speakers at the symposium demonstrated that this analogy has continued to inform research on plant cell biology. Joseph Atabekov (Moscow State University, Russia) discussed viral functions involved in intercellular movement. Specifically, TMV spreads from cell to cell through plasmodesmata until it reaches the vascular system, which mediates long-distance transport. The spread of TMV through the vasculature may be a primarily passive process, occurring with the flow of photoassimilates. In contrast, cell-to-cell movement requires specific interactions between virus components and plasmodesmata. Such interactions are mediated in the case of TMV by the MP (Figure 1), which acts to increase plasmodesmal permeability and to facilitate transport of viral genomic RNA through these enlarged channels. Surprisingly, TMV can also mediate the movement of unrelated viruses, such as potato leaf roll luteovirus (PLRV), which are normally limited to the host phloem (Atabekov and Taliansky, 1990).

Research into the ability of TMV to promote virus nonspecific cell-to-cell movement has focused on the TMV MP, and this research has contributed broadly to our understanding of plant intercellular communication. Vitaly Citovsky (State University of New York, Stony Brook) described his initial discovery that the TMV MP specifically binds to single-stranded nucleic acids, and he surmised that the MP directly attaches to viral RNA in a sequence-nonspecific manner to facilitate plasmodesmal transport. Citovsky went on to report the recent identification of a 38-kD tobacco cell wall protein (p38) which specifically binds to TMV MP. Two MP domains are involved in p38 recognition, and these regions were previously shown to be required for viral movement and gating of plasmodesmata (Gafny et al., 1992; Waigmann et al., 1994). He also reported that the effect of MP on plasmodesmal permeability is negatively regulated by phosphorylation of serine and threonine residues near its C terminus (Citovsky et al., 1993). Citovsky's presentation closed with the description of a recessive single gene mutation in Arabidopsis, termed *vsm1*, which blocks the systemic spread of TMV (Lartey et al., 1998), and he suggested that this mutant might be useful in efforts to elucidate the general mechanisms of intercellular molecular movement in plants.

Bill Dawson (University of Florida, Lake Alfred) offered an overview of attempts by researchers during the past century to identify the causes of symptoms associated with TMV infection. Dawson recalled that Beijerinck first observed that TMV produces a disease of chloroplasts, a finding that was pursued by F.C. Bawden in the 1930s when he linked chlorosis to specific stages of TMV infection and disease (Bawden, 1939). Dawson intrigued symposium participants with his ability to predict the onset of systemic vein clearing with an accuracy of about 20 min. Dawson's 'magic box' showed that, counter to virologists' intuition, vein clearing may not be a direct consequence of virus infection, but rather can result from the rapid physiological signaling that precedes viral invasion of the upper leaves. These results may relate to molecular genetic studies showing that the mosaic phenotype (Atkinson and Matthews, 1970) is dependent on the amino acid composition of the TMV replicase proteins (Lewandowski and Dawson, 1993). Dawson's presentation illustrated the perspective gained by combining the traditional physiological and pathological concepts in TMV biology with the insights realized from modern cellular and molecular approaches.

Beijerinck's Legacy: Biotechnology

Speaking of more modern approaches, Roger Beachy (The Donald Danforth Plant Science Center, St. Louis, MO) discussed the recent contributions that TMV research has made toward elucidating the mechanism of pathogen-derived host protection, a topic that illustrates the reciprocating interests of basic science on the one hand and commercial incentives on the other. Beachy reminded the audience that resistance against TMV in CP-expressing transgenic plants seems to be a protein-mediated process in which the cytoplasmic accumulation of CP subunits somehow affects the disassembly of incoming TMV particles (Bendahmane et al., 1997). Through a

MEETING REPORT

sophisticated combination of reverse genetics and powerful computing procedures, experiments in Beachy's laboratory are beginning to shed light on the intricate structural interactions involved in CP-mediated protection. More generally, the achievements of his laboratory since the 1980s have paved the way for numerous other strategies for engineering pathogen-derived resistance. These biotechnological innovations have in turn advanced our understanding of cosuppression and transgene silencing, events that have provided headaches (and opportunities) for many researchers working with transgenic plants.

Barbara Baker (United States Department of Agriculture–Plant Gene Expression Center, Albany, CA) offered an update on studies of the *N* gene, which provides gene-for-gene resistance against TMV. The significance of this discovery became apparent upon the demonstration that the transgenic introduction of the *N* gene into tomato plants confers the ability to activate a TMV-specific hypersensitive response (Whitham et al., 1996). Although *N* is a single gene in tobacco, Baker reported recent results suggesting that alternative splicing of the *N* transcript plays a role in mediating resistance, and she outlined the ongoing evaluations of postulated molecular interactions between the product of the *N* gene and the viral replicase protein.

The ascendance of biotechnology in the 1980s and 1990s has reoriented TMV research toward commercial application, as Wilson pointed out. For example, the TMV Ω-leader sequence has been utilized to substantially enhance the translation of certain transgenes (Gallie et al., 1987). From Wilson's subsequent summary of recent progress in the development of virus vectors, it became apparent that TMV is playing a leading role in the exploration of various strategies to use viruses as vectors for expressing foreign genes in plants. Tom Turpen (Biosource Technologies, Vacaville, CA) echoed the current interest in TMV not only as a model system, but also as a commercial vector. The high titer of TMV in infected plants and the renowned stability of the virus contribute to its attractiveness for use in large-scale production of highly valued compounds. Turpen reported that Biosource has used TMV to produce therapeutic human enzymes of high purity and specific activity, and that pilot tests are underway in new biomass processing facilities in Kentucky for the harvesting of commercially valuable proteins from field-grown tobacco plants. One could not fail to reflect upon the socioeconomic and political implications of "milking tobacco leaves for human enzymes," especially when it is happening in "tobacco country".

These recent biotechnological advances encompass the entire history of research on TMV, from its discovery in an agricultural context to its most modern practical applications. Lute Bos (Wageningen Agricultural University, The Netherlands) argued that one century ago the convergence of agricultural concerns over "mosaic infected" tobacco and the emerging germ theory of disease gave Beijerinck's discovery of TMV its resounding scientific impact (Bos, 1995). At the beginnings of virology as a discipline, TMV helped researchers define what a virus was, and throughout the twentieth century, experimentation on this model virus has led scientists to a greater understanding of both life and disease.

Current developments in TMV research are equally auspicious. The exploitation of the TMV genome, the molecular mining of natural reservoirs of genetic resistance, and the use of viruses as molecular tools, represent promising and potentially very powerful avenues of investigation. Even in this modern era of accelerating appreciation for molecular mechanisms, TMV research maintains its status as a pioneering endeavor to advance our understanding of biology.

Angela N.H. Creager
Program in History of Science
Princeton University
Princeton, NJ 08544-1017
creager@princeton.edu

Karen-Beth G. Scholthof
Department of Plant Pathology
and Microbiology
Texas A&M University
College Station, TX 77843-2132
kbgs@acs.tamu.edu

Vitaly Citovsky
Department of Biochemistry
and Cell Biology
State University of New York
at Stony Brook
Stony Brook, NY 11794-5215
vitaly.citovsky@sunysb.edu

Herman B. Scholthof
Department of Plant Pathology
and Microbiology
Texas A&M University
College Station, TX 77843-2132
herscho@acs.tamu.edu

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MEETING REPORT

ogy" edited by K.-B.G. Scholthof, J.G. Shaw, and M. Zaitlin is scheduled for publication in the spring of 1999 by the American Phytopathological Society Press (St. Paul, MN).

REFERENCES

Abel, P.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.

Anderer, F.A., Uhlig, H., Weber, E., and Schramm, G. (1960). Primary structure of the protein of tobacco mosaic virus. Nature **186,** 922–925.

Atabekov, J.G., and Taliansky, M.E. (1990). Expression of a plant virus-coded transport function by different viral genomes. Adv. Virus Res. **38,** 201–248.

Atkinson, P.H., and Matthews, R.E.F. (1970). On the origin of dark green tissue in tobacco leaves infected with tobacco mosaic virus. Virology **40,** 344–356.

Bawden, F.C., Pirie, N.W., Bernal, J.D., and Fankuchen, I. (1936). Liquid crystalline substances from virus-infected plants. Nature **138,** 1051–1055.

Bawden, F.C. (1939). Plant Viruses and Virus Diseases. (Leiden, The Netherlands: Chronica Botanica Company).

Beachy, R.N., Zaitlin, M., Bruening, G., and Israel, H.W. (1976). A genetic map for the cowpea strain of TMV. Virology **73,** 498–507.

Beijerinck, M.W. (1898). Über ein contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. Verh. K. Akad. Wet. Amsterdam **65,** 3–21. (Translation published in English as Phytopathological Classics No. 7 [1942], American Phytopathological Society, St. Paul, MN.)

Bendahmane, M., Fitchen, J.H., Zhang, G., and Beachy, R.N. (1997). Studies of coat protein–mediated resistance to tobacco mosaic tobamovirus: Correlation between assembly of mutant coat proteins and resistance. J. Virol. **71,** 7942–7950.

Bos, L. (1995). The embryonic beginning of virology: Unbiased thinking and dogmatic stagnation. Arch. Virol. **140,** 613–619.

Bloomer, A.C., Champness, J.N., Bricogne, G., Staden, R., and Klug, A. (1978). Protein disk of tobacco mosaic virus at 2.8 Å resolution showing the interactions within and between subunits. Nature **276,** 362–368.

Butler, P.J.G. (1984). The current picture of the structure and assembly of tobacco mosaic virus. J. Gen. Virol. **65,** 253–279.

Caspar, D.L.D. (1956). Radial density distribution in the tobacco mosaic virus particle. Nature **177,** 928.

Caspar, D.L.D. (1960). The structural stability of tobacco mosaic virus. Trans. N.Y. Acad. Sci. **22,** 519–521.

Citovsky, V., Knorr, D., Schuster, G., and Zambryski, P. (1990). The P30 movement protein of tobacco mosaic virus is a single-stranded nucleic acid binding protein. Cell **60,** 637–647.

Citovsky, V., McLean, B.G., Zupan, J.R., and Zambryski, P. (1993). Phosphorylation of tobacco mosaic virus cell-to-cell movement protein by a developmentally regulated plant cell wall–associated protein kinase. Genes Dev. **7,** 904–910.

Crick, F.C., and Watson, J.D. (1956). Structure of small viruses. Nature **177,** 473–475.

Dawson, W.O., Beck, D.L., Knorr, D.A., and Grantham, G.L. (1986). cDNA cloning of the complete genome of tobacco mosaic virus and production of infectious transcripts. Proc. Natl. Acad. Sci. USA **83,** 1832–1836.

Deom, C.M., Oliver, M.J., and Beachy, R.N. (1987). The 30-kilodalton gene product of tobacco mosaic virus potentiates virus movement. Science **237,** 389–394.

Domingo, E., and Holland, J.J. (1994). Mutation rates and rapid evolution of RNA viruses. In The Evolutionary Biology of Viruses, S.S. Morse, ed (New York: Raven Press), pp. 161–184.

Eriksson-Quensel, I.-B., and Svedberg, T. (1936). Sedimentation and electrophoresis of the tobacco-mosaic virus protein. J. Am. Chem. Soc. **58,** 1863–1867.

Fraenkel-Conrat, H., and Williams, R.C. (1955). Reconstitution of active tobacco mosaic virus from its inactive protein and nucleic acid components. Proc. Natl. Acad. Sci. USA **41,** 690–698.

Fraenkel-Conrat, H. (1956). The role of the nucleic acid in the reconstitution of active tobacco mosaic virus. J. Am. Chem. Soc. **78,** 882–883.

Fraenkel-Conrat, H., and Singer, B. (1957). Virus reconstitution. II. Combination of protein and nucleic acid from different strains. Biochim. Biophys. Acta **24,** 540–548.

Fraile, A., Escriu, F., Aranda, M.A., Malpica, J.M., Gibbs, A.J., and García-Arenal, F. (1997). A century of tobamovirus evolution in an Australian population of *Nicotiana glauca.* J. Virol. **71,** 8316–8320.

Franklin, R.E. (1955). Structure of tobacco mosaic virus. Nature **175,** 379–381.

Franklin, R.E. (1956). Location of the ribonucleic acid in the tobacco mosaic virus particle. Nature **177,** 928–930.

Franklin, R.E., Klug, A., and Holmes, K.C. (1956). X-ray diffraction studies of the structure and morphology of tobacco mosaic virus. In Ciba Foundation Symposium on The Nature of Viruses, G.E.W. Wolstenholme and E.C.P. Millar, eds (London, UK: Churchill Press), pp. 39–55.

Gafny, R., Lapidot, M., Berna, A., Holt, C.A., Deom, C.M., and Beachy, R.N. (1992). Effects of terminal deletion mutations on function of the movement protein of tobacco mosaic virus. Virology **187,** 499–507.

Gallie, D.R., Sleat, D.E., Watts, J.W., Turner, P.C., and Wilson, T.M.A. (1987). The 5′-leader sequence of tobacco mosaic virus RNA enhances the expression of foreign gene transcripts in vitro and in vivo. Nucleic Acids Res. **15,** 3257–3273.

Gierer, A., and Schramm, G. (1956). Infectivity of ribonucleic acid from tobacco mosaic virus. Nature **177,** 702–703.

Gierer, A., and Mundry, K.-W. (1958). Production of mutants of tobacco mosaic virus by chemical alteration of its ribonucleic acid in vitro. Nature **182,** 1457–1458.

Goelet, P., Lomonossoff, G.P., Butler, P.J.G., Akam, M.E., Gait, M.J., and Karn, J. (1982). Nucleotide sequence of tobacco mosaic virus RNA. Proc. Natl. Acad. Sci. USA **79,** 5818–5822.

Heinlein, M., Epel, B.L., Padgett, H.S., and Beachy, R.N. (1995). Interaction of tobamovirus movement proteins with the plant cytoskeleton. Science **270,** 1983–1985.

Hunter, T.R., Hunt, T., Knowland, J., and Zimmern, D. (1976). Messenger RNA for

MEETING REPORT

the coat protein of tobacco mosaic virus. Nature **260,** 759–764.

Ivanowski, D. (1892). Über die Mosaikkrankheit der Tabakspflanze. Bull. Acad. Imp. Sci. St. Petersb., Nouv. Sér. **3:35,** 67–70. (Translation published in English as Phytopathological Classics No. 7 [1942], American Phytopathological Society, St. Paul, MN.)

Jackson, A.O., Zaitlin, M., Siegel, A., and Francki, R.I.B. (1972). Replication of tobacco mosaic virus. III. Viral RNA metabolism in separate leaf cells. Virology **48,** 655–665.

Joshi, S., Pleij, C.W.A., Haenni, A.L., Chapeville, F., and Bosch, L. (1983). The nature of the tobacco mosaic virus intermediate length RNA-2 and its translation. Virology **127,** 100–111.

Kausche, G.A., Pfankuch, E., and Ruska, H. (1939). Die Sichtbarmachung von pflanzlichem Virus im Übermikroskop. Naturwissenschaften **27,** 292–299.

Kay, L.E. (1998). A book of life? How the genome became an information system and DNA a language. Persp. Biol. Med. **41,** 504–526.

Lartey, R.T., Ghoshroy, S., and Citovsky, V. (1998). Identification of an *Arabidopsis thaliana* mutation (*vsm1*) that restricts systemic movement of tobamoviruses. Mol. Plant-Microbe Interact. **11,** 706–709.

Lauffer, M.A., Ansevin, A.T., Cartwright, T.E., and Brinton, C.C. (1958). Polymerization—depolymerization of tobacco mosaic virus protein. Nature **181,** 1338–1339.

Lewandowski, D.J., and Dawson, W.O. (1993). A single amino acid change in tobacco mosaic virus replicase prevents symptom production. Mol. Plant-Microbe Interact. **6,** 157–160.

Mayer, A. (1886). Über die Mosaikkrankheit des Tabaks. Landwn. Vers-Stnen. **32,** 451–467. (Translation published in English as Phytopathological Classics No. 7 [1942], American Phytopathological Society, St. Paul, MN.)

McLean, B.G., Zupan, J., and Zambryski, P. (1995). Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. Plant Cell **7,** 2101–2114.

Meshi, T., Ishikawa, M., Motoyoshi, F., Semba, K., and Okada, Y. (1986). In vitro transcription of infectious RNAs from full-length cDNAs of tobacco mosaic virus. Proc. Natl. Acad. Sci. USA **83,** 5043–5047.

Namba, K., Pattanayek, R., and Stubbs, G. (1989). Visualization of protein–nucleic acid interactions in a virus. Refined structure of intact tobacco mosaic virus at 2.9Å resolution by x-ray fiber diffraction. J. Mol. Biol. **208,** 307–325.

Nishiguchi, M., Motoyoshi, F., and Oshima, N. (1978). Behaviour of a temperature sensitive strain of tobacco mosaic virus in tomato leaves and protoplasts. J. Gen. Virol. **39,** 53–61.

Ohno, T., Takamatsu, N., Meshi, T., Okada, Y., Nishiguchi, M., and Kiho, Y. (1983). Single amino acid substitution in 30K protein of TMV defective in virus transport function. Virology **131,** 255–258.

Palukaitis, P., and Zaitlin, M. (1986). Tobacco mosaic virus: Infectivity and replication. In Plant Viruses. Vol. 2. The Rod-Shaped Plant Viruses, M.H.V. Van Regenmortel and H. Fraenkel-Conrat, eds (New York: Plenum Press), pp. 105–131.

Pelham, H.R.B. (1978). Leaky UAG termination codon in tobacco mosaic virus RNA. Nature **272,** 469–471.

Stanley, W.M. (1935). Isolation of a crystalline protein possessing the properties of tobacco mosaic virus. Science **81,** 644–645.

Tsugita, A., Gish, D.T., Young, J., Fraenkel-Conrat, H., Knight, C.A., and Stanley, W.M. (1960). The complete amino acid sequence of the protein of tobacco mosaic virus. Proc. Natl. Acad. Sci. USA **46,** 1463–1469.

Waigmann, E., Lucas, W., Citovsky, V., and Zambryski, P. (1994). Direct functional assay for tobacco mosaic virus cell-to-cell movement protein and identification of a domain involved in increasing plasmodesmal permeability. Proc. Natl. Acad. Sci. USA **91,** 1433–1437.

Watanabe, Y., Emori, Y., Ooshika, I., Meshi, T., Ohno, T., and Okada, Y. (1984). Synthesis of TMV-specific RNAs and proteins at the early stage of infection in tobacco protoplasts: Transient expression of the 30 K protein and its RNA. Virology **133,** 18–24.

Watson, J.D. (1954). The structure of tobacco mosaic virus. I. x-Ray evidence of a helical arrangement of sub-units around the longitudinal axis. Biochim. Biophys. Acta **13,** 10–19.

Whitham, S., McCormick, S., and Baker, B. (1996). The *N* gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato. Proc. Natl. Acad. Sci. USA **93,** 8776–8781.

Wilson, T.M.A. (1984). Cotranslational disassembly of tobacco mosaic virus in vitro. Virology **137,** 255–265.

Wolf, S., Deom, C.M., Beachy, R.N., and Lucas, W.J. (1989). Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. Science **246,** 377–379.

Wu, X., and Shaw, J.G. (1996). Bidirectional uncoating of the genomic RNA of a helical virus. Proc. Natl. Acad. Sci. USA **93,** 2981–2984.

Wu, X., and Shaw, J.G. (1997). Evidence that a viral replicase protein is involved in the disassembly of tobacco mosaic virus particles in vivo. Virology **239,** 426–434.

Zaitlin, M. (1998). The discovery of the causal agent of the tobacco mosaic virus disease. In Discoveries in Plant Biology, S.D. Kung and S.F. Yang, eds. (Hong Kong: World Scientific Publishing Co. Ltd.), pp. 105–110.