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Milestones in the research on tobacco mosaic virus

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Beijerinck's (1898) recognition that the cause of tobacco mosaic disease was a novel kind of pathogen became the breakthrough which eventually led to the establishment of virology as a science. Research on this agent, tobacco mosaic virus (TMV), has continued to be at the forefront of virology for the past century.

After an initial phase, in which numerous biological properties of TMV were discovered, its particles were the first shown to consist of RNA and protein, and X-ray diffraction analysis of their structure was the first of a helical nucleoprotein. In the molecular biological phase of research, TMV RNA was the first plant virus genome to be sequenced completely, its genes were found to be expressed by cotranslational particle disassembly and the use of subgenomic mRNA, and the mechanism of assembly of progeny particles from their separate parts was discovered. Molecular genetical and cell biological techniques were then used to clarify the roles and modes of action of the TMV non-structural proteins: the 126 kDa and 183 kDa replicase components and the 30 kDa cell-to-cell movement protein. Three different TMV genes were found to act as avirulence genes, eliciting hypersensitive responses controlled by specific, but different, plant genes. One of these (the *N* gene) was the first plant gene controlling virus resistance to be isolated and sequenced. In the biotechnological sphere, TMV has found several applications: as the first source of transgene sequences conferring virus resistance, in vaccines consisting of TMV particles genetically engineered to carry foreign epitopes, and in systems for expressing foreign genes.

TMV owes much of its popularity as a research model to the great stability and high yield of its particles. Although modern methods have much decreased the need for such properties, and TMV may have a less dominant role in the future, it continues to occupy a prominent position in both fundamental and applied research.

Keywords: history of TMV; plant virology; tobacco mosaic virus; tobamovirus; virology milestones

1. INTRODUCTION

Research on tobacco mosaic virus (TMV) has played a leading role in the development of virology for more than a century. Table 1 gives the chronology of 50 important advances in research involving TMV. Factually, and in many instances also conceptually, almost all broke new ground in plant virology, and many did so in general virology too. The aim of this article is to relate this series of key discoveries to the more specialized papers which follow, and which comprise a unique collection of perspectives of the past, present and future.

2. EARLY STUDIES

Research on the disease caused by TMV goes back at least as far as the work of Adolf Mayer, a German who, in 1876, became Director of the Agricultural Experiment Station at Wageningen in The Netherlands. In 1886 he described the retardation of growth, decreased yield, and the accompanying curling and brittleness of tobacco leaves that made them unsuitable for cigar making. It was he who named this syndrome 'tobacco mosaic', because of the pattern of light and dark green areas on the foliage (Mayer 1886). His most important discovery, however, was that the condition was infectious and could be induced by sucking juice from diseased leaves into a fine glass capillary, which was then plunged into a leaf vein of a healthy plant. Symptoms did not develop in the inoculated leaf but, after about ten days, they appeared in the youngest leaves on the plant and continued to develop in all leaves produced subsequently. Mayer looked for a pathogen but failed to find one, although he showed that the infectivity of leaf extracts was destroyed by prolonged heating at 80 °C.

The next important discovery was made by Dmitrii Ivanowski, who studied tobacco mosaic disease in the Crimea and, in 1892, published his seminal paper in which he reported that extracts of mosaic-affected leaves remained infective after passage through Chamberland filter candles designed to be impenetrable to bacteria (Ivanowski 1892). However, despite checking the quality of his filter candles by showing that the filtrates, although favourable for the growth of bacteria, remained entirely unchanged for several months, he seems to have failed to make the conceptual advance needed to recognize that he was dealing with a pathogen of a novel type.

This was the achievement of Martinus Willem Beijerinck (1898), Professor of Bacteriology in the Technical School of Delft. He repeated the filtration experiments (figure 1), showed that the disease agent could diffuse into agar gel and concluded that it multiplied in the plant but not *in vitro*. He named it a *contagium vivum fluidum* and considered it to be either soluble or very minute. He also obtained evidence that it multiplied best in actively growing tissue in which

Table 1. *Fifty milestones in research on tobacco mosaic virus (TMV)*

year	milestone
1886	experimental transmission by inoculation with sap (Mayer)
1892	passage through Chamberland filter (Ivanowski)
1898	distinction from bacterial pathogens; *contagium vivum fluidum* (Beijerinck)
1928	specific antigen in infected plants (Purdy)
1929	cross-protection between virus strains (McKinney)
1929	local lesion infectivity assay (Holmes)
1932	flow birefringence of TMV-containing sap (Takahashi & Rawlins)
1934	single dominant gene controlling the hypersensitive reaction (HR) (Holmes)
1935	proteinaceous nature of purified virus particles (Stanley)
1936	particles are anisometric and consist of ribonucleoprotein (Bawden & Pirie)
1936	regular substructure in particles (Bernal & Fankuchen)
1939	rod-shaped particles observed by electron microscopy (Kausche *et al.*)
1947	particle amino acid composition differs among virus strains (Knight)
1952	molecular weight of coat protein (CP) subunits (Harris & Knight)
1954	helical structure of the virus particle (Watson)
1955	particle reassembly from viral CP and RNA (Fraenkel-Conrat & Williams)
1956	infectivity of purified viral RNA (Gierer & Schramm)
1956	control of CP specificity by viral RNA (Fraenkel-Conrat)
1956	location of RNA in the virus particle (Caspar; Franklin)
1957	arrangement of CP subunits and RNA in the helical particle (Franklin *et al.*)
1958	chemical mutagenesis of TMV RNA (Gierer & Mundry)
1960	amino acid sequence of CP (Anderer *et al.*; Tsugita *et al.*)
1962	evidence for non-overlapping triplet genetic code (Tsugita; Wittmann)
1963	antipeptide virus-neutralizing antibodies (Anderer)
1969	protoplast-infection system (Takebe & Otsuki)
1970	host PR protein production associated with HR (Gianinazzi *et al.*; Van Loon & Van Kammen)
1971	role of CP disks in particle assembly (Butler & Klug)
1972	low molecular weight viral RNA (Jackson *et al.*)
1976	translation of CP from subgenomic RNA (Hunter *et al.*)
1977	nucleotide sequence of origin of particle assembly (Zimmern)
1977	mechanism of particle assembly (Butler *et al.*; Lebeurier *et al.*)
1978	expression of 183 kDa protein by readthrough of 126 kDa ORF (Pelham)
1978	evidence for viral cell-to-cell movement function (Nishiguchi *et al.*)
1982	complete nucleotide sequence of genomic RNA (Goelet *et al.*)
1984	cotranslational disassembly of virus particles (Wilson)
1984	protein antigenicity linked to segmental mobility (Westhof *et al.*)
1986	particle structure determined at 3.6 Å resolution (Namba & Stubbs)
1986	infectious RNA from full-length cDNA clones (Dawson *et al.*; Meshi *et al.*)
1986	transgenic resistance mediated by the CP gene (Powell Abel *et al.*)
1986	packaging of recombinant RNA in pseudovirus particles (Sleat *et al.*)
1986	expression of foreign epitope on TMV CP (Haynes *et al.*)
1987	TMV as a gene vector (Takamatsu *et al.*)
1987	enzymic nature of PR proteins (Legrand *et al.*)
1987	avirulence function of the CP gene (Saito *et al.*)
1987–1990	movement protein functions: targeting plasmodesmata (Tomenius *et al.*); increasing plasmodesmal size exclusion limit (Wolf *et al.*); binding to single-stranded nucleic acid (Citovsky *et al.*)
1994	sequence of tobacco *N* gene (Whitham *et al.*)
1996	coreplicational disassembly of particles from 3′-end (Wu & Shaw)
1997	polymerase complex containing two viral proteins and a host protein (Osman & Buck)

cell division was still in progress, and that long distance movement within the plant was via phloem tissue. The scene was now set for the recognition of a new category of infectious agents, at first termed filterable viruses, but later known simply as viruses. These early developments in virology are discussed in detail by Bos (this issue).

3. BIOLOGICAL PROPERTIES

During the first part of the 20th century, various biological properties of TMV were studied. The virus was found to have a considerable host range, especially among solanaceous species. Also, mutant forms were isolated from the yellow spots that occur occasionally on systemically infected leaves. These mutants seemed to arise spontaneously, and their appearance was not simply the result of selection from a pre-existing mixture of variants (Jensen 1933). However, leaves systemically infected with a typical isolate resisted infection by the mutants, a result which led McKinney (1929) to recognize the phenomenon of cross-protection between virus strains. No such cross-protection occurred between isolates of distinct

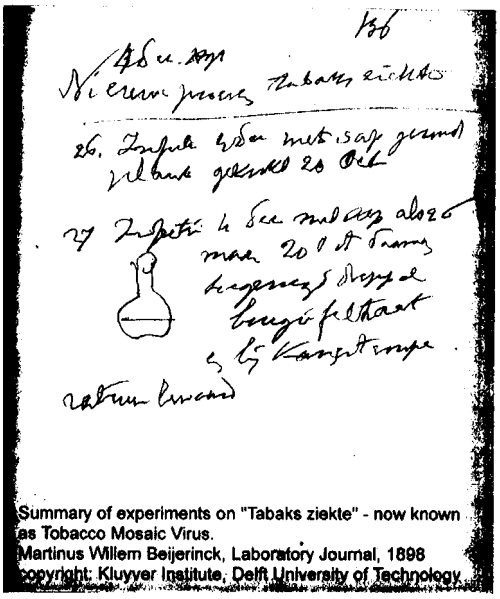

Figure 1. Page from M. W. Beijerinck's laboratory notebook recording details of the TMV filtration experiment. (Courtesy L. A. Robertson).

Figure 2. Necrotic lesions induced by TMV in an inoculated leaf of *Nicotiana glutinosa* (Scottish Crop Research Institute).

viruses. More recently, cross-protection has been exploited to protect crops, such as tomatoes, from infection with the more pathogenic strains of tomato mosaic virus (which is closely related to TMV) by pre-inoculating them with mild strains (Rast 1972). This so-called mild strain protection has been used extensively in Japan.

TMV invades almost all tissues of a plant, except the root and shoot apices and the reproductive cells. Once infected, a plant remains so for the rest of its life but the infection is usually not passed on through seed to progeny seedlings. Infection of a low percentage of progeny seedlings is caused chiefly by the abrasion of young seedlings with the virus-contaminated seed testa during transplanting (Broadbent 1965).

TMV proved to be transmitted readily by manual inoculation, by grafting and by contact, and to be physically and biologically tough. Temperatures above 90°C for ten minutes were needed to abolish the infectivity of virus-containing sap. However, quantitative assays of infectivity were crude until Holmes (1929), working with TMV and *Nicotiana glutinosa*, introduced the local lesion-assay method (figure 2). He also showed that the local lesion reaction confined infection to the inoculated leaf and that it was controlled by a single dominant gene (Holmes 1934), which subsequently was transferred from *N. glutinosa* to *N. tabacum*. TMV-resistant tobacco cultivars were thereby produced.

Little progress was made in determining the nature of the infectious agent in sap from tobacco mosaic-affected plants until about 30 years after Beijerinck's (1898) paper. It could not be seen by light microscopy in infective filtrates and it could not be cultured outside the plant. Then the evidence began to accumulate. Purdy (1928) found a specific antigen in infective sap, Takahashi & Rawlins (1932) discovered that such sap showed anisotropy of flow when examined in plane-polarized light, suggesting the presence of elongated particles, and Vinson & Petre (1931) partially purified the infectious agent by precipitation with lead acetate.

4. IMPACT OF BIOCHEMICAL AND STRUCTURAL ANALYSIS

At a time when enzymes were first being purified, and were proving to be proteins, a similar approach was applied to TMV by Stanley (1935, 1936), working in the United States. He obtained preparations containing needle-like 'crystals' (later shown to be paracrystals) that were 100-fold more infective than crude leaf extracts and contained an antigen specific to infected plants. From analyses of these preparations, Stanley concluded that the 'crystals' contained protein, and he likened TMV to 'an autocatalytic protein which, for the present, may be assumed to require the presence of living cells for multiplication' (1935, p. 645).

Stanley (1936) failed to detect any phosphorus in his TMV preparations and thought that they consisted only of protein. In contrast, Bawden *et al*. (1936), and Bawden & Pirie (1937) showed that highly infective, purified preparations of TMV particles contained phosphorus and carbohydrate, and concluded that they consisted of about 5% RNA and 95% protein. Bawden & Pirie (1937) also found that purified particle preparations showed anisotropy of flow (figure 3) and that suspensions containing more than 1.6% of the nucleoprotein were spontaneously birefringent. This was interpreted as indicating that the virus had rod-shaped particles which aggregated end-to-end when purified. Concurrent X-ray diffraction data showed that the rods had a diameter of 152 Å and a structural repeat along their length at about 68 Å (Bawden *et al*. 1936; Bernal & Fankuchen 1937), the first clue to the substructure of a virus particle.

This series of papers from the United States and British laboratories stimulated great interest and much further work. For example, particles of other viruses were purified. Those of tomato bushy stunt virus contained 15–20% RNA and could form true crystals (Bawden & Pirie 1938). The rod-like shape of TMV particles was confirmed by the electron micrographs of

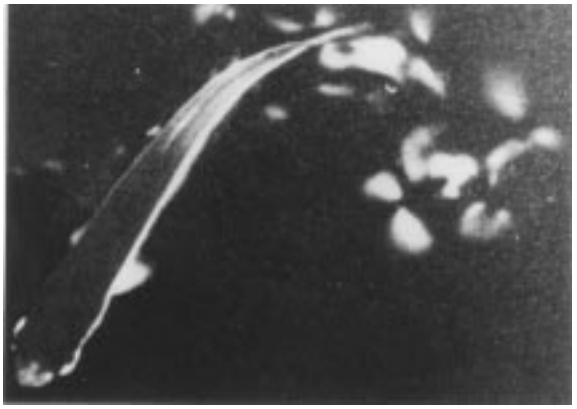

Figure 3. Wake of a goldfish swimming in a dilute suspension of TMV and viewed in plane-polarized light. The fluid becomes birefringent where the rod-shaped particles are orientated by the flow movements. (Bawden *et al.*, 1936; courtesy of the Institute of Arable Crops Research, Rothamsted).

Kausche *et al.* (1939), the first to be taken of any virus. Later the particles were found to have a modal length of about 300 nm. By further X-ray studies, Watson (1954) deduced that the protein in TMV particles consisted of subunits, packed in a helical array, which repeated every 68 Å (the value from Bawden *et al.* (1936)), representing $3n+1$ subunits in three turns of the helix. Watson could say little about the location of the RNA in TMV particles but suggested that it was internal. However, the technique of isomorphous replacement, in which single atoms of lead or mercury were coupled to individual protein subunits in the TMV particle, allowed further details of its structure to be determined. The particle was found to have 49 subunits in three turns of the helix, and its radial density distribution showed the particle to be a hollow tube (Caspar 1956) with the RNA embedded in the protein helix at a radius of 40 Å (Franklin 1956). Thus the TMV particle was the first helically constructed nucleoprotein to be understood structurally and the first in which the position of the nucleic acid could be defined precisely (Klug, this issue). More recent studies on TMV particle structure and the current, more detailed understanding of it are described by Stubbs (this issue).

As a result of these advances, ideas about the nature of viruses changed in the 1940s and 1950s to the point where the view that they had greater affinities with components of normal cells than with pathogenic microorganisms was becoming more popular. For example, Bawden & Pirie (1953, p. 4) published a lengthy review entitled 'Virus multiplication considered as a form of protein synthesis'. However, they noted in the tailpiece, wisely as it turned out, that their argument 'accounts for only part of the virus particle. With as much, or as little, justification (they wrote) we could also have argued that nothing is known that sharply distinguishes virus multiplication from the synthesis of nucleic acid. We chose protein synthesis, because, although ideas about it are still nebulous, they have attained more form than ideas about nucleic acid synthesis'.

Another breakthrough was made at about the time the main features of the TMV particle structure were determined. First, Schramm & Zillig (1955) found that protein subunits, isolated from TMV particles, could be reaggregated in the absence of RNA to form rod-shaped particles that were noninfectious. However, when Fraenkel-Conrat & Williams (1955) mixed noninfectious TMV protein, prepared from TMV particles by alkali treatment, with apparently non-infectious RNA, prepared by disrupting TMV particles with detergent, an infectious nucleoprotein with the properties of TMV particles was produced. This astounding result attracted great interest, for it seemed that an agent endowed with genetic continuity had been produced by associating two kinds of non-infectious molecules. This interpretation was, however, short-lived because the following year Gierer & Schramm (1956) showed that purified preparations of TMV RNA, obtained by treating TMV particles with phenol, were indeed infective but that the infectivity was labile. It was abolished by low concentrations of ribonuclease that had no effect on TMV particles, but was largely unaffected by treatment with TMV antiserum or by ultracentrifugation, each of which removed TMV particles from inocula. However, probably because of the extracellular and intracellular lability of TMV RNA, its specific infectivity is much lower than that of TMV particles. Thus when TMV particles were reconstituted by Fraenkel-Conrat & Williams (1955), infectivity was not created *de novo,* but infectious TMV RNA was protected from inactivation. Fraenkel-Conrat (1956) too began to find infectivity in his TMV RNA preparations and made the further important observation that virus particles reconstituted from protein of the ribgrass strain of TMV and RNA of the common strain were infectious, and that progeny virus particles contained exclusively the protein of the common strain. Thus TMV RNA was the carrier of infectivity and of genetic information controlling protein specificity. These important discoveries stimulated a great new wave of TMV research, rapidly leading to further advances in knowledge. One of the most important of these was that genetic mutants of TMV could be produced by treating TMV RNA with nitrous acid (Gierer & Mundry 1958), which converts cytosine to uracil, and adenine to hypoxanthine (which behaves like guanine). The fascinating recollections of these events by Fraenkel-Conrat & Singer can be found in their contribution to this issue. The formation and characterization of aggregates of TMV particle protein is discussed by Klug (this issue), and the reconstitution of TMV particles is reviewed in detail by Butler (this issue).

5. THE MOLECULAR BIOLOGICAL APPROACH

As molecular biology developed, the emphasis in TMV research changed from the study of viral protein to that of viral RNA. Indeed, when the genetic code was being deciphered, data obtained from chemically produced TMV mutants with altered particle (coat) proteins (CPs) provided the key evidence that nucleotide triplets encoding individual amino acids do not overlap (Tsugita 1962; Wittmann 1962).

Some years later, TMV RNA was the first plant viral genome to be sequenced completely (Goelet *et al.* 1982). However, this achievement was the culmination of efforts in several laboratories (Okada, this issue). Moreover, the existence and functions of several TMV gene products

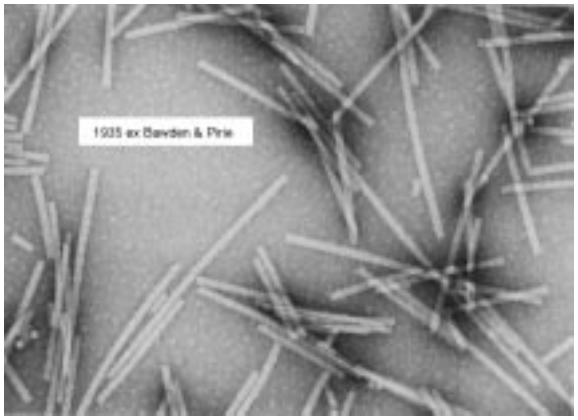

Figure 4. Electron micrograph of a preparation of TMV particles, purified by F. C. Bawden & N. W. Pirie in 1935 and examined after storage for 50 years. The negatively stained particles are 18 nm in diameter.

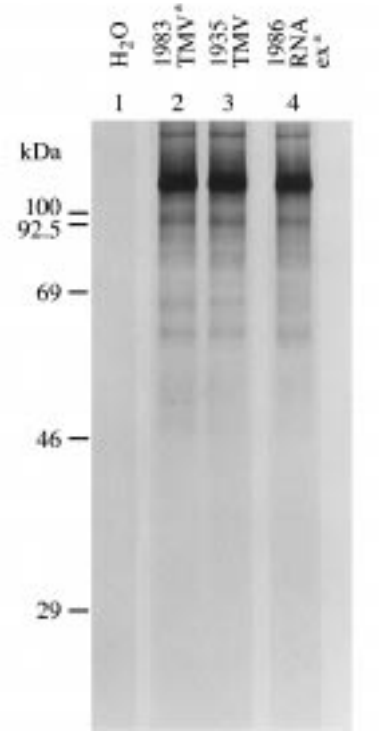

Figure 5. Cotranslational disassembly of fresh and stored TMV particles in a rabbit reticulocyte cell-free translation system. Lanes show electrophoretic analysis of the products of translation reactions containing (left to right): water (control), fresh (1983) TMV particles, 51-year-old TMV particles (purified by F. C. Bawden & N. W. Pirie in 1935), TMV RNA (prepared in 1986 from TMV particles purified in 1983). (T. M. A. Wilson, unpublished results obtained in 1986).

had already been deduced before the complete sequence was determined (Zaitlin, this issue). Nevertheless, knowledge of the complete genomic nucleotide sequence allowed the position of each gene, and the amino acid sequence of each gene product, to be defined precisely. It also led to comparisons of mutants and to elucidation of the nature and roles of various control signals.

Aided by the development of protoplast infection systems (Takebe & Otsuki 1969), which enabled a single synchronous cycle of virus replication to be studied in isolation (in contrast to the many overlapping cycles that coexist in intact plant tissue), concepts of virus multiplication changed again. From being likened to the synthesis of cell components (Bawden & Pirie 1953), TMV replication was now found to consist of translation of the viral RNA to produce the TMV replicase, which in turn synthesized minus-sense and then plus-sense copies of the RNA. Translation of the viral CP gene then followed, leading to the assembly of progeny virus particles from full-length genomic RNA and the CP.

Demonstration that a low molecular weight RNA found in TMV-infected cells is the subgenomic messenger RNA for the viral CP was the first example of its kind in plant virology (Hunter *et al*. 1976). Within a few years the mechanisms of expression of all four recognized TMV genes were worked out (Okada, this issue).

For the virus replication cycle to start, it seemed likely that the release of TMV RNA from virus particles would be a prerequisite. Yet TMV particles were known to be extraordinarily stable *in vitro* (figure 4). Remarkably, the disassembly of TMV particles is initiated when they become associated with ribosomes, leading to translation of the 5′-terminal open reading frame, a process termed cotranslational disassembly (Wilson 1984; figure 5). The details of this process are described by Shaw (this issue). The subsequent steps of RNA replication (Buck, this issue), synthesis of TMV CP, and assembly of progeny TMV particles (Butler, this issue), complete the TMV replication cycle (Okada, this issue).

Due to of the ease with which particles of TMV and its relatives in the tobamovirus group (Harrison *et al*. 1971) could be purified, complete genomic nucleotide sequences have been determined for at least 12 tobamovirus species. Comparison of these sequences has revealed that all tobamoviruses are more closely related to one another than to any other virus. Lineages within the group can be correlated largely with those of their plant hosts, suggesting that tobamoviruses have differentiated and coevolved with their hosts (Gibbs, this issue). In addition, the stability of its particles has allowed infectious tobacco mild green mosaic tobamovirus to be recovered from 100-year-old herbarium specimens. Comparison of such isolates from *Nicotiana glauca* in Australia with present-day isolates showed that the virus has not evolved appreciably during the past century. This work, and evidence on the wider relationships of tobamoviruses, are summarized by Gibbs (this issue).

6. CONFLUENCE OF VIROLOGY, MOLECULAR GENETICS AND CELL BIOLOGY

One problem not resolved by earlier studies on TMV replication was how the virus spreads from cell to cell. Plasmodesmata in uninfected tissue are too small to allow the passage of such large structures as TMV particles or TMV RNA. The discovery that TMV possesses a gene which mediates cell-to-cell movement (Nishiguchi *et al*. 1978; Ohno *et al*. 1983), and that the encoded movement protein (MP) can be detected serologically in plasmodesmata (Tomenius *et al*. 1987; see also figure 6), has led to much further research on the mechanisms involved (Atabekov *et al*., this issue; Citovsky, this issue); yet another example of TMV research leading the field.

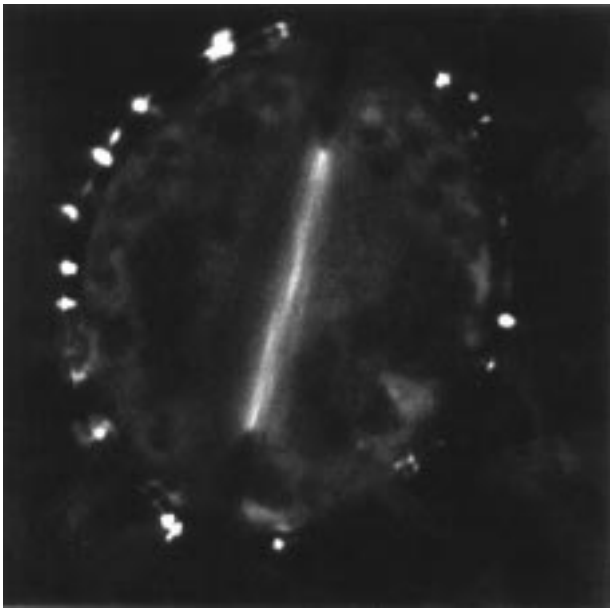

Figure 6. Distribution of TMV 30 K movement protein, fused to the jellyfish green fluorescent protein, in cells of tobacco plants systemically infected with a recombinant TMV isolate carrying the gene for the fusion protein. The protein has accumulated at points in the cell wall (bright spots), thought to represent plasmodesmata, where the two stomatal guard cells adjoin other epidermal cells. The guard cells are 37 μm long. Photographed by confocal laser scanning microscopy. (Courtesy K. J. Oparka & R. N. Beachy).

Two technical developments were crucial aids to analyse the functions and mechanisms of action of MP and other viral gene products. The first involved the cloning, as cDNA, of a full-length viral genomic nucleotide sequence from which infectious viral RNA could be transcribed. TMV was the second plant virus for which this was achieved (Dawson *et al.* 1986; Meshi *et al.* 1986). This advance led to the application of reverse genetics, in which individual viral genes were rendered inactive or modified in predetermined ways and the effect(s) on viral processes ascertained. In this way, the functions (multiple in some instances) of gene products, or of domains within these proteins, could be analysed. The second important development was the introduction of visible markers of gene expression, so enabling gene products to be located in cells and tissues. Initially, the most popular of these markers was *E. coli* β-glucuronidase (GUS), but this has now been largely superseded by GFP (the jellyfish green, fluorescent protein), which can be observed under ultraviolet light without killing the infected tissue, and can be seen at high resolution in individual cells by confocal laser scanning microscopy (figure 6).

Application of these different approaches, and the injection of cells with other fluorescent molecules of known molecular weight has, for example, led to the demonstration that TMV MP increases the plasmodesmal size exclusion limit, and shown the time-course of its accumulation at plasmodesmata and its ability to bind single-stranded RNA (Citovsky, this issue). Also, although we are still far from understanding how viruses induce disease symptoms in plants, and how one TMV strain may induce a green mosaic whereas a second causes a yellow mosaic and a third infects symptomlessly, the combined application of reverse genetics and cell biological techniques should greatly assist in finding the answers (see Dawson, this issue).

As already mentioned, TMV infection is confined to the necrotic local lesions in tobacco or tomato plants that carry the appropriate genes. This hypersensitive reaction (HR) is an important form of resistance in commercial crops. Earlier work showed that the HR was accompanied by an accumulation of numerous host proteins, dubbed pathogenesis-related proteins (PR proteins; Gianinazzi *et al.* 1970; Van Loon & Van Kammen 1970), several of which proved to be enzymes (Legrand *et al.* 1987). Phenotypically similar kinds of HR are controlled by distinct single host genes. Molecular genetic evidence has shown that each host gene is activated by a specific viral protein which, according to the host gene in question, is either the TMV polymerase, MP or CP (Okada, this issue; Dawson, this issue; Erickson *et al.*, this issue). The cognate TMV gene acts as an avirulence gene and key motifs in the viral protein have been identified that elicit the HR. Clearly, tobacco and tomato have evolved a variety of defences against TMV.

The best known of the tobacco genes controlling the HR, the *N* gene, was the first viral resistance gene to be isolated and sequenced (Whitham *et al.* 1994). Its expression is elicited by the TMV 126 kDa replicase protein. The *N* gene product contains several domains with recognisable affinities to structures with known functions, and is similar in design to the products of genes that control HR to fungal or bacterial pathogens in other plant species (Erickson *et al.*, this issue).

7. BIOTECHNOLOGY

The discovery, in the 1980s, of a method to insert foreign nucleotide sequences into the genome of tobacco plants, led to attempts to create virus-resistant plants by genetic engineering. In the first of these to be successful, it was found that transformation with a DNA copy of the TMV CP gene, furnished with suitable transcriptional promoter and terminator sequences, produced a TMV-resistant plant genotype (Powell Abel *et al.* 1986). The transgenic tobacco plants were substantially more resistant to TMV infection than control plants; but, if infected, they accumulated normal concentrations of TMV. Moreover, this resistance was largely ineffective against TMV RNA inocula, suggesting that particle disassembly was being inhibited (Register & Beachy 1988). This CP-mediated approach has since proved to be applicable to many positive-sense RNA viruses of plants. Further details of how the resistance operates and how it can be maximized are given by Beachy (this issue). This breakthrough led to a spate of similar approaches in which virus resistance was sought by transformation with a variety of virus-related nucleotide sequences. For example, a particularly effective procedure was discovered by using the 3′-terminal third of the TMV 183 kDa gene (Zaitlin, this issue), but the resistance is probably too virus strain-specific to be useful on its own for protecting commercial crops against tobamoviruses.

In a very different kind of biotechnology, TMV particles were used as surface carriers of foreign peptides so that the modified virus could be used as a vaccine to

induce the production of antibodies against the unrelated agent. As early as 1963, Anderer found that neutralizing antibodies could be produced against a peptide derived from the TMV CP, and later Westhof *et al*. (1984) showed that high segmental mobility in the polypeptide chain of the TMV CP was important for antigenicity (Van Regenmortel, this issue). In one example of the use of such information, efforts were made to produce an anti-malarial vaccine by expressing an epitope of a malarial agent on the particle surface of genetically engineered TMV (Turpen, this issue).

TMV can also be used as a vector for foreign genes. For instance, Takamatsu *et al*. (1987) expressed chloramphenicol acetyl transferase (CAT) from a cDNA construct in which the CAT gene replaced the CP gene. More recently, TMV isolates in which the CP gene, and hence the ability to cause systemic infection, are retained have been engineered to express foreign genes. Some of these virus isolates are being cultured in inoculated plants on a field scale for commercial production of valuable biological materials (Turpen, this issue).

Finally, the value of plant viruses, and of TMV in particular, as a source of discrete functional modules for genetic engineering was emphasized by Wilson (1989). For instance, in addition to the above applications, TMV nucleotide sequences can be used to produce pseudovirus particles in which the RNA comprises a foreign mRNA coupled to the TMV origin-of-assembly sequence, and may also carry the TMV 5′-terminal untranslated leader sequence (Ω′), in such a way that the chimaeric mRNA is packaged in and protected by the TMV CP (Sleat *et al*. 1986), but is liberated and expressed efficiently in inoculated cells. The TMV RNA leader sequence was one of the first two translational enhancers shown to function *in vitro* and *in vivo* in several systems and with many coding sequences (Gallie *et al*. 1987).

8. CONCLUDING COMMENTS

In this article, we have attempted to link the more detailed descriptions of individual aspects of TMV research that are covered in other contributions. Some of the accompanying papers are historical accounts, mostly written by those who made key discoveries. These, and the different viewpoints they express, make fascinating reading. At the other extreme are papers describing some of the latest advances, and show that TMV remains at the cutting edge of important aspects of plant virology and biotechnology.

We have also related the development and contribution of TMV research to successive, largely technique-led, phases of research in the biological sciences more generally. TMV owes its prominence in virus research largely to the stability of its particles and to the relatively large amounts in which they are produced in plants. Increasingly, these needs can be circumvented by modern procedures, which are making possible advanced research on a variety of other plant viruses that have their own distinctive properties. Therefore, although it will remain an important model in virus and virus-related research, TMV seems unlikely to contribute as greatly to advances and applications of virology in the next century as it has done in the century now ending. In this sense, the papers that follow are perhaps more timely and significant than is suggested merely by the celebration of a centenary.

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