ANNUAL REVIEWS

Annual Review of Virology

Tobacco Mosaic Virus and the History of Molecular Biology

Angela N.H. Creager

Department of History, Princeton University, Princeton, New Jersey; USA;
email: creager@princeton.edu

Annu. Rev. Virol. 2022. 9:39–55

First published as a Review in Advance on June 15, 2022

The *Annual Review of Virology* is online at virology.annualreviews.org

https://doi.org/10.1146/annurev-virology-100520-014520

Keywords

tobacco mosaic virus, Wendell Stanley, James Watson, Rosalind Franklin, crystallography, genetic code

Abstract

The history of tobacco mosaic virus (TMV) includes many firsts in science, beginning with its being the first virus identified. This review offers an overview of a history of research on TMV, with an emphasis on its close connections to the emergence and development of molecular biology.

INTRODUCTION

There are many reasons to revisit the history of research on tobacco mosaic virus (TMV), beginning with the fact that it was the first virus to be identified and so marks the start of the field of virology. However, not every original example of a new biological category becomes a well-studied object in its own right (1, 2). As virology took off in the early twentieth century, TMV did become one of the best-studied viruses and remained at the forefront of the field. It was used to elucidate basic knowledge about the nature of viruses and served as a model system in biomedicine as well as agriculture, where it had emerged. The fact that the first recognized virus came from plants—although animal viruses were rapidly identified—meant that virology was, from the outset, highly comparative (3).

This review focuses on how and why TMV became so influential in the life sciences, paying special attention to its stand-out role in molecular biology. Literature on the origins of molecular biology often privileges bacteriophage and the contributions of the Phage Group (4). Yet early work with TMV inspired Max Delbrück and other early molecular biologists to take up the study of bacteriophages. Moreover, TMV itself became a prominent model system for understanding the molecular nature of heredity and the relationship between proteins and nucleic acids (5). Notably, some of the main scientists involved in elucidating the double-helical structure of DNA were also studying TMV, which became a tool for cracking the genetic code.

TOBACCO MOSAIC VIRUS FROM MOTTLED LEAVES TO MACROMOLECULE

In the late nineteenth century, not long after the germ theory of disease transformed medicine, scientists began puzzling over a new class of infectious agents that did not adhere to Koch's postulates. These observations first emerged in the lucrative crop tobacco, a South American plant that entered the Old World through Spanish networks of trade with New World indigenous peoples before becoming domesticated throughout Europe (6, 7). In 1876 Adolf Mayer, a German agricultural chemist and director of the Agricultural Experimental Station at Wageningen, the Netherlands, began studying a mottling disease affecting tobacco (**Figure 1**). He named it tobacco mosaic disease and showed that the juice extracted from leaves of diseased plants could be used to infect healthy plants (8). This experimental transmission suggested a bacterium or fungus, but repeated microscopical examination of these extracts did not turn up an organism, nor could Mayer cultivate the pathogen in the laboratory. Dmitri Ivanovskii in Russia followed up Mayer's investigations; in 1892 he reported that the infectivity of sap from mosaic-diseased tobacco plants remained infectious after passing through a bacteria-retaining filter (9). Independently, Dutch microbiologist Martinus Willem Beijerinck made the same observation (10). Unlike Ivanovskii, Beijerinck claimed that the agent of tobacco mosaic disease was not a miniscule bacterium but something fundamentally different: a *contagium vivum fluidum*, or contagious living fluid (11). This set in motion a 25-year debate over what viruses were, liquid or particulate (11, 12).

Of these three microbiologists who first studied tobacco mosaic, only Beijerinck employed the older, somewhat generic term virus in his paper. (On the term virus, see Reference 13, appendix A.) He associated it with this new class of submicroscopic pathogens. In 1898, two animal pathogens, for foot-and-mouth disease (14) and rabbit myxomatosis (15), were also found to pass through sterilizing filters. Agents for African horse-sickness (16), fowl plague (17–19), and yellow fever (20) soon joined the growing list of filterable viruses, which were largely defined in the negative: infectious agents that could not be removed by sterilizing filters, seen in microscopes, or grown in cell-free culture. In 1900, John M'Fadyean declared that viruses were obligatory parasites (16). This was recognized as a central feature of viruses by the 1920s, despite some continued attempts

Figure 1

Photograph of a tobacco mosaic virus–infected tobacco plant. Photo courtesy of Karen-Beth G. Scholthof.

to culture these filter-passing pathogens in cell-free media (21). The tools of bacteriology defined viruses but proved to be of little assistance in isolating them.

Although many of the viruses being documented were from animals or humans, TMV remained especially tractable for laboratory research, as it was highly transmissible and had a long shelf life in filtered sap (22). Plant scientists launched productive lines of investigation into TMV's infectivity, genetics, immunochemistry, and the development of host resistance (23). The Boyce Thompson Institute for Plant Research, opened in 1924, quickly became the epicenter of TMV work in the United States. There, Louis Kunkel led a research group on mosaic diseases that included Francis Holmes, who developed a quantitative method to determine TMV infectivity, the local lesion assay (24–26). Helen Purdy raised rabbit antibodies against TMV and demonstrated they could neutralize infection (27, 28). Another of Kunkel's researchers, Carl Vinson, tried to isolate TMV chemically using lead acetate but could not obtain pure preparations (29, 30).

In 1932, Kunkel moved to the Rockefeller Institute for Medical Research as head of its new Division of Plant Pathology in Princeton. He brought some staff along but hired a better chemist than Vinson, Wendell M. Stanley. Also located at the Princeton branch of the Rockefeller Institute was chemist John Northrop, who was crystallizing digestive enzymes beginning with pepsin (31, 32). Stanley treated extracts of TMV-infected leaves with Northrop's purified pepsin and trypsin; the reduction in viral infectivity strongly suggested the plant virus was a protein. He drew on Northrop's ammonium sulfate methods of precipitation, in conjunction with Holmes's local lesion assay, to purify the virus. In 1935, Stanley published a paper in *Science* announcing that he had obtained protein crystals of TMV (33, 34) (**Figure 2**). His paper was covered on the front page of the *New York Times* under the headline "Crystals Isolated at Princeton Believed Unseen Disease Virus" (5, 35).

Frederick C. Bawden and Norman W. Pirie in Britain repeated Stanley's isolation and challenged his claims (36, 37). TMV was found to contain nucleic acid (RNA) as well as protein, and its needle-shaped precipitates were not true crystals. However, they also confirmed the value of

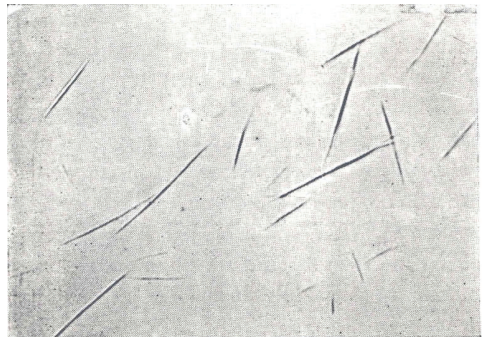

Figure 2

A photomicrograph of Wendell Stanley's (para)crystalline tobacco mosaic virus, magnified 675 times. This was reproduced widely in Stanley's publications. Figure reproduced from the Franklin Institute of the state of Philadelphia, Report No. 3187. January 14, 1948. Page 8. Plates I and II, showing magnified images of the tobacco mosaic virus. Wendell M. Stanley papers, BANC MSS 78/18c, The Bancroft Library, University of California, Berkeley.

his chemical approach. Stanley's subsequent research yielded a wealth of information about the physical properties of TMV and other plant viruses (38–41). The impact of Stanley's 1935 paper, by suggesting that a virus might be as chemically simple as table salt, went beyond his specific findings (42). Hermann J. Muller referred to Stanley's isolated virus as "a certain kind of gene" (43, p. 213). Max Delbrück was inspired by the crystallization of TMV to see viruses as key to solving the "riddle of life" (44, p. 1312). TMV research was too chemically oriented for Delbrück's taste, but he found in bacteriophage a system suited to quantitative analysis of virus genetics (4, 45).

Stanley remains best known for his crystallization of TMV, yet a chance discovery made with Ralph Wyckoff in the 1930s had a greater material impact on virus research. Wyckoff, a biophysicist at the Rockefeller Institute, was analyzing samples of Stanley's TMV in the ultracentrifuge and observed that when he centrifuged clear juice from mosaic-diseased tobacco plants at 25,000 rpm, a pellet of fibrous material sedimented to the bottom of the tube. When analyzed in a microscope, this pellet was seen to be composed of needle-shaped crystals. Wyckoff and his coworker Robert Corey soon published evidence that these centrifuge-produced crystals were indistinguishable from the crystalline material Stanley prepared by chemical means (46). Thus, the ultracentrifuge, which had been called by one *New York Times* writer "the new whirligig of science" (47), could be used not only to analyze viruses but also to prepare them in pure form. This was a major development for research on the many viruses too fragile for chemical extraction. Wyckoff and collaborators used the ultracentrifuge to isolate potato X virus, tobacco ring spot virus, cucumber mosaic virus, tobacco necrosis virus, and papilloma virus from rabbits (39, 48, 49). In all of these studies, TMV provided a key point of reference for treating viruses as pure macromolecules—these other viruses quite literally followed TMV into the ultracentrifuge (5, 50).

This biophysical work on TMV suggested that not only proteins but also massive viruses were homogeneous molecular entities rather than heterogeneous aggregates of smaller chemical units (51). However, some plant pathologists were dubious. In their first long publication on TMV, Bawden and Pirie argued that Stanley's "isolated product . . . has become aggregated" (37, p. 306). Stanley, responding privately to Bawden early in 1938, made clear that he accepted their chemical corrections and their concerns about the harsh precipitation techniques required for crystallization, but would not concede that the homogenous, rod-shaped macromolecules were artifacts of centrifugation (52).

TMV was the first virus visualized in the electron microscope, and the results confirmed those from ultracentrifugation. The micrographs of TMV published by Gustav A. Kausche, Edgar Pfannkuch, and Helmut Ruska in 1939 revealed discrete rod-shaped particles of 330 × 15 nanometers (53). Two years later, Stanley and Thomas Anderson obtained their own quite clear pictures on an RCA electron microscope, with rods 280 nanometers long (54). In 1946, Stanley shared the Nobel Prize in Chemistry with Northrop and James Sumner for preparing enzymes and viruses in pure form.

TOBACCO MOSAIC VIRUS STRUCTURE

During World War II, results from laboratories in Great Britain and Germany called into question Stanley's suggestion that TMV was a single covalently bonded macromolecule. Based on analysis of X-ray diffraction patterns, crystallographers J.D. Bernal and Isidore Fankuchen saw evidence for smaller repeating units in the virus structure (55). In Dahlem (on the outskirts of Berlin), TMV was being investigated in a new workshop for virus research established jointly by the Kaiser Wilhelm Institutes for Biochemistry (headed by Adolf Butenandt) and Biology (headed by Alfred Kühn and Fritz von Wettstein) (56–59). (Evacuated to Tübingen in 1943, after the war, this workshop became the basis for the Max Planck Institute for Virus Research.) Georg Melchers obtained a sample of TMV in 1938 from Stanley with which to begin studies in Dahlem (60). In 1943, his colleague Gerhard Schramm reported that exposing TMV to alkaline conditions produced, beyond normal TMV, two dissociated viral pieces of similar size: one a nucleoprotein, and one a nucleic acid–free protein (61). This last species appeared to be homogeneous; a molecular weight of 360,000 Da was calculated. More surprisingly, a subsequent drop in pH caused this protein to form a large rod-shaped macromolecule similar in its size and properties to intact TMV (62). This TMV-resembling rod, however, was not infective and so had lost the property of self-reproduction.

Into the 1950s, Schramm continued investigating the properties of various alkali-produced fragments of TMV. Yet his portrayal of TMV as composed of discrete subunits received surprisingly little attention from biologists in the United States and the United Kingdom. In *The Double Helix*, James D. Watson attributed the distrust of Schramm's finding to the war:

> It was inconceivable to most people that the German beasts would have permitted the extensive experiments underlying his claims to be routinely carried out during the last years of a war they were so badly losing. It was all too easy to imagine that the work had direct Nazi support and that his experiments were incorrectly analyzed (63, p. 112).

Schramm was, in fact, a member of the Nazi Party and even affiliated with the SS (Schutzstaffel), although his TMV research had no obvious relation to the German war effort (64). Schramm and Stanley exchanged letters and reprints in 1947, by which time Stanley had moved to the University of California, Berkeley, where he established the Virus Laboratory. Yet Stanley did not take seriously the physiological significance of Schramm's TMV subunits until a serendipitous experiment in 1951 made it undeniable.

At that time, J. Ieuan Harris was working on the amino acid composition of adrenocorticotropic hormone with Choh Hao Li, a well-known hormone biochemist at Berkeley. Harris was searching for a control for his carboxypeptidase digest of the hormone, and so came to Stanley's laboratory to procure some TMV, which the Virus Laboratory workers considered to be a single polypeptide 350,000 amino acids long (65, 66). But rather than yielding one mole of end-product amino acid from TMV, as Harris expected from the control, the carboxypeptidase split off more than 3,000 molecules of threonine per virus (67, 68)!

This surprising result both alerted members of the Virus Laboratory to the existence of structural subunits of TMV and motivated them to pay greater attention to Schramm's work, as he

was also reporting 3,000 virus subunits from chemical analysis of the N terminus. When Heinz Fraenkel-Conrat joined Stanley's unit in 1952, he focused on determining the number and chemical structure of the subunits. Despite the apparent agreement between number of amino-terminal residues identified in Tübingen and the number of carboxy-terminal residues found in Berkeley, the rival groups engaged in an open feud over the number of TMV protein subunits and end groups (69, 70).

Even so, underlying their dispute was a new consensus in the conceptualization of virus as an assembly of identical polypeptides and RNA. Schramm's early reporting of subunits of TMV had been correct. He dubbed his nucleic acid–free TMV fragments (now of molecular weight 120,000) A-protein, for the alkaline conditions producing them (69). As he observed, if the virus polypeptides had identical carboxy-terminal and amino-terminal end groups, then most likely all of the other amino acids in the polypeptide were also identical (71). The race was on to characterize the TMV protein subunit just as Frederick Sanger was analyzing insulin (72, 73). But whereas insulin had 21 amino acids in one chain and 30 in the other, the TMV coat protein (CP) turned out to contain 158 amino acids. As Heinz Fraenkel-Conrat recalled, "the era of protein sequencing was upon us" (66, p. 311).

None of these biochemists yet possessed an appreciation for the key role of the nucleic acid in TMV, even when they uncovered suggestive evidence (74). Despite the 1952 Hershey-Chase experiment on T4 bacteriophage, with its demonstration that the DNA was the hereditary component of the virus (75), members of Stanley's Virus Laboratory doubted that the TMV RNA participated in the virus's genetics (76). For his part, in 1954 Schramm suggested that TMV mutations were not alterations in the nucleic acid or protein composition but rather conformational changes (71).

THE DNA PROTAGONISTS AND TOBACCO MOSAIC VIRUS

Watson, who began to work on the structure of TMV in 1952, held an altogether different view of the role of nucleic acids in heredity. Every schoolchild now learns of his collaboration with Francis Crick in elucidating the double-helical structure of DNA. But that was not either's official project during Watson's time in Cambridge. While Crick was supposed to be finishing a doctoral thesis on X-ray diffraction methods for proteins (77), Watson was trying to determine the structure of TMV (63). Building on William Cochran, Crick, and Vladimir Vand's helical diffraction theory (78), Watson realized that some X-ray diffraction spots of TMV that had puzzled Bernal and Fankuchen could be explained if TMV were in a helical configuration.

Working with Roy Markham at the Molteno Institute, Watson took his own X-ray diffraction patterns of TMV paracrystals. They confirmed his hunch. Based on his new data, he argued that TMV was a helix repeating every three turns with a period of 68 Å. He also suggested that the viral RNA was in the center of this helix, analogous to its placement in two (roughly) spherical viruses, turnip yellow mosaic virus and T2 bacteriophage (79). Watson's paper on TMV was submitted to *Biochimica et Biophysica Acta* the week before his famous note with Crick on DNA's double-stranded structure appeared in *Nature* (80). Needless to say, Watson's familiarity with helical diffraction theory had proven very useful in grasping the full significance of Rosalind Franklin's "Photograph 51" (81). Like TMV, DNA was paracrystalline, requiring fiber diffraction techniques to resolve.

Franklin herself began working on TMV in 1953 in J.D. Bernal's laboratory at Birkbeck, after leaving DNA behind at King's College. [The virus would not have been unfamiliar; Maurice Wilkins had been working on TMV as well as DNA while Franklin was at King's (82).] Working with a TMV sample from Markham's Cambridge lab, Franklin obtained her first diffraction patterns on the virus late that year. After experimenting with different humidity conditions (as

she had with DNA), she obtained remarkably clear patterns in the spring of 1954, with more than 300 distinct maxima (83). After being invited to a Gordon Conference to take place that fall, she wrote Stanley, inquiring if she might visit the Virus Laboratory while in the United States and give a lecture there (83–85). She arrived at Berkeley in October 1954 after visiting Caltech. She arranged to obtain samples of purified TMV from Fraenkel-Conrat and C. Arthur Knight at Berkeley, including a heavy-metal derivative. As Franklin told Stanley, she relied on the Berkeley TMV preparation as the standard for her diffraction work (86). She was also in contact with Gerhard Schramm, whose TMV-derived A-protein she wanted to analyze using X-ray diffraction (5). Barry Commoner had isolated a similar constituent of the TMV protein rod that he called B8 protein. Franklin visited him in his St. Louis laboratory while in the United States, and they collaborated on a subsequent publication analyzing his material (87).

Both at Caltech and at an earlier stop at Woods Hole, Franklin met with Watson. As Watson wrote to Crick after their conversations, he and Leslie Orgel were "trying to make more sense out of TMV (promoted by Rosie's visit—very amiable!)" (88). Having moved to Caltech in September 1953, Watson was continuing to do structural studies on nucleic acids by collaborating with Alex Rich on RNA. TMV was one of several sources of their RNA samples. Using X-ray diffraction, they found similar patterns from RNAs from plant viruses, calf thymus, and yeast. It was difficult for them, however, to discern whether the RNA structures were helical or not, or to make biological sense of the results. Plant viral RNA lacked the 1:1 complementary base ratios that characterized DNA, which seemed to necessitate a different mechanism of replication if the RNA was hereditary material (89). In a letter to Delbrück, Watson described the emerging picture as "queer and paradoxical" and declared their project at a standstill (90). Rich left for a postdoc at the National Institutes of Health, where he started doing X-ray diffraction studies of TMV (91).

Late in 1954 Franklin drafted a paper on her results with TMV, including a speculative model of the placement of subunits (78). After circulating it to several colleagues, including Watson and Crick, she published her preliminary structure in *Nature* early in 1955. It confirmed Watson's depiction of TMV as a helix "most satisfactorily," while updating his estimate of the number of subunits per turn with her clearer data (92, p. 380). Her patterns suggested extensive grooves along the surface of the helix, a feature consistent with evidence that the virus could be chemically modified without disrupting its structure, or in some cases even its infectivity. She also determined the distribution of density from the center of the helix, which led her to postulate that the nucleic acid was situated close to the protein subunits, not along the center like a candle wick, as electron micrographs of TMV fragments had suggested and as Watson had speculated.

Donald Caspar came to Caltech as a postdoctoral fellow in 1954, rekindling Watson's interest in TMV (78). Caspar had studied TMV structure for his doctoral dissertation at Yale, using heavy-atom isomorphous replacement (with lead) to determine the radial density of the cylindrical TMV particle. He continued this work after arriving at Caltech. His results suggested that the TMV rod was hollow, independently confirming Franklin's model. Caspar and Franklin began corresponding about their similar results. They made plans for him to visit her laboratory late in 1955, where Aaron Klug had already joined Franklin working on TMV structure. By comparing repolymerized A-protein (which was nucleic acid free) with intact TMV, Franklin and Caspar were able to resolve that the RNA was about 40 Å from the center, a result published as consecutive articles in *Nature* (93, 94).

Two months before Franklin and Caspar's papers appeared, but informed by these ongoing results, Crick and Watson had published a theoretical paper in *Nature* on virus structure. They generalized that all small viruses are composed of identical protein subunits surrounding nucleic acid and that simple construction rules constrained the possible number of virus structures (95). Building on Caspar's diffraction results with tomato bushy stunt virus (which were published in

Figure 3

Photograph of a tobacco mosaic virus (TMV) model constructed for the International Exhibit in Brussels in 1958. This large model was subsequently displayed in a stairwell at the Cambridge Laboratory of Molecular Biology. The cable exposed under the egg-shaped protein subunits represents the strand of viral RNA. This model represents only a short segment of a complete TMV particle, not its full length. Photo copyright Gregory J. Morgan. Reproduced with permission.

an accompanying paper), they argued that all spherical viruses exhibited cubic symmetry (96, 97). In a subsequent presentation that spring at the Ciba Foundation Symposium on the Nature of Viruses, Crick and Watson argued that the virtue of many identical protein subunits was that one relatively short gene could encode the entire viral shell (98). The underlying simplicity of virus structures prompted Crick's witticism that "Any child could make a virus" (22, p. 303).

At that same Ciba Symposium, Franklin and her Birkbeck coworkers unveiled an updated structural model of TMV. They corrected the number of subunits per three turns of the helix to its current value of 49. At 40 Å from the center, the RNA was embedded in the helically arranged protein subunits (99). They argued for a single strand of RNA per virus particle, although as Watson observed in the discussion that followed, the X-ray diffraction data could also be consistent with multiple strands (as he and Caspar had postulated in unpublished work). The TMV structure Franklin and her coworkers developed was presented at the 1958 Brussels World's Fair; sadly, just as that exhibition opened in April, she succumbed to cancer (78) (**Figure 3**).

RECONSTITUTION AND THE ROSETTA STONE

Alongside the advances in knowledge of TMV's structure were tremendous strides in biochemical work on the virus. In the spring of 1955, Fraenkel-Conrat and electron microscopist Robley

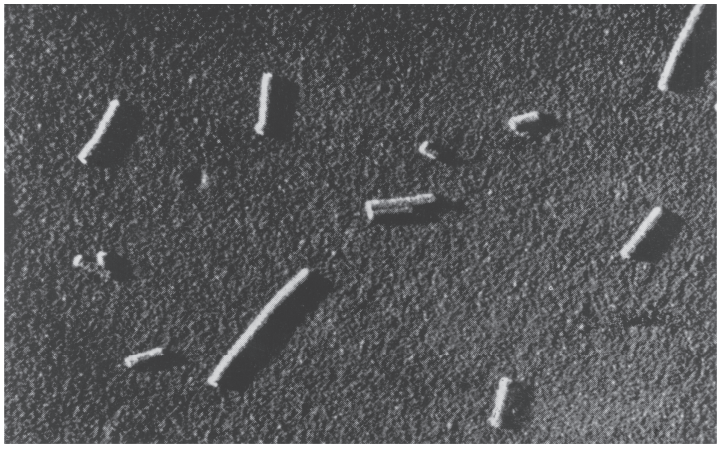

Figure 4

Electron micrographs of particles of reconstituted tobacco mosaic virus (TMV). Their morphology is identical to that of normal TMV, except for a greater proportion of short particles. The long rod in this field is 300 nanometers long, the same as a full-length virus particle (magnified 60,000 times). Photo reprinted from Reference 100. Reproduced courtesy of Bea Singer Fraenkel-Conrat and Robley C. Williams, Jr.

Williams published a striking result: Purified viral protein and purified RNA from TMV could be recombined to yield infective particles, at about 1% of the activity of the original virus (100). This reconstitution experiment showed that TMV could be assembled from its components in a test tube, the first such demonstration for a virus. Purified protein alone, they reported, was not infectious, and neither was the purified RNA, except at very high concentrations, a result they attributed to contamination by intact virus. Thus, the biochemical reassembly resulted in the production of infectious virus from apparently inactive components (**Figure 4**).

This experiment garnered a great deal of popular attention (5). Newspapers carried the United Press–syndicated story under the headline "Scientists Create Life in Test Tube" (101). Waldemar Kaempffert, science editor for the *New York Times*, included the reconstitution of TMV as one of the four most important scientific events of the year (102). *Collier's* magazine informed the public of these developments under the title "Now—MAN-MADE VIRUS—First Step in Controlling Heredity?" According to this journalist, by producing a chemical that could "reproduce—as if it were a living thing," the experiment was "a stunning moment in the long history of man's attempt to unravel the secret of life itself" (103, p. 75).

Fraenkel-Conrat and coworker Bea Singer then began making reconstituted hybrids of different TMV strains. In every case where the nucleic acid from one strain was combined with the protein from another strain, the progeny virus was identical to the parent strain from which the nucleic acid had been derived (**Figure 5**). A particularly striking example was achieved by mixing TMV protein with RNA from Holmes ribgrass (HR), a strain whose protein is quite distinct from garden-variety TMV in both amino acid composition and antigenicity. The reconstituted hybrid virus showed none of the distinctive antigenic properties of HR, but when infected into tobacco it produced the virus progeny with protein indistinguishable from the HR strain (104, 105). The genetic role of the RNA was unambiguous, and Fraenkel-Conrat now believed the infectiousness of RNA was not due to contamination with intact virus (104).

Three weeks after Fraenkel-Conrat submitted a brief communication on the role of TMV RNA to the *Journal of the American Chemical Society* (104), Alfred Gierer and Schramm independently submitted a more substantial letter to *Nature* titled "Infectivity of Ribonucleic Acid from Tobacco Mosaic Virus" (106). This publication provided compelling evidence that TMV RNA was

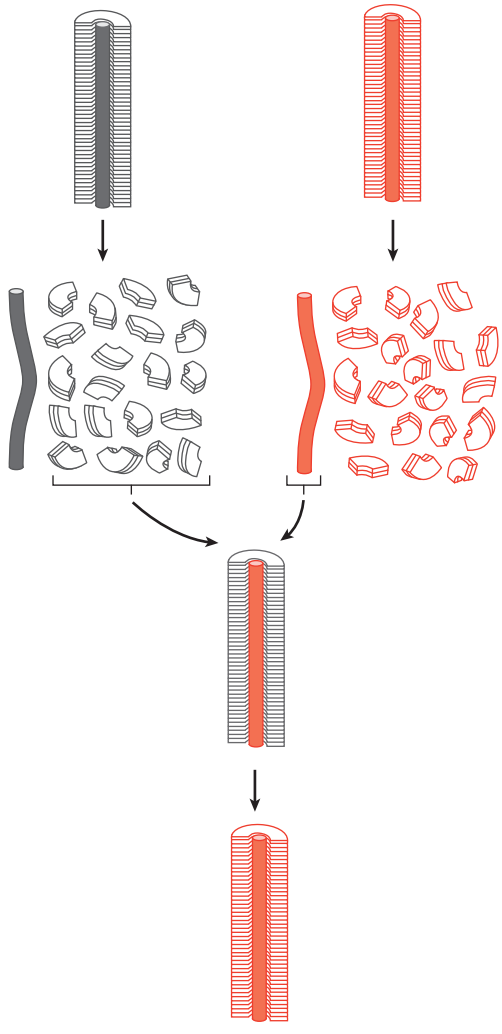

Figure 5

Schematic diagram showing the hereditary role of RNA in reconstituted hybrid tobacco mosaic virus. Two strains of virus (shortened cross sections, *top row*, strains represented by two different colors) were broken down into their constituent nucleic acids and protein subunits (*second row*), and the protein of one strain was allowed to recombine with the nucleic acid (RNA) of the other (*third row*). The progeny of this hybrid (*fourth row*) shows the (*red*) protein resulting from its parental nucleic acid. Figure and caption adapted from Reference 145.

infectious by itself, although at a much lower rate than that of native virus. Their method of phenol extraction of the nucleic acid, adapted from the repertoire of techniques used in polysaccharide biochemistry, produced a larger RNA (12–18S) than had been previously identified. Although the infectivity of RNA was only 2% that of native virus, controls showed that the TMV RNA they isolated contained only 0.02% native TMV protein. "We are thus led to conclude that the infectivity is due to the nucleic acid itself" (106, p. 702). The following year Gierer demonstrated that even a single disruption of the complete RNA chain could cause a complete loss of infectivity (107). He also showed TMV RNA to be single stranded.

While the rival groups in Germany and the United States contested priority, it is perhaps more notable that neither group had tested the infectivity of RNA in the prior 20 years (108). According to Bryan Harrison, Bawden and Pirie had tested isolated TMV RNA for infectivity much earlier, with negative results. Harrison presumed the 50-mile distance between Pirie's biochemistry lab and Bawden's greenhouse meant that the RNA was degraded before it reached the tobacco plants (109). Bawden and Pirie were certainly the most vociferous skeptics of the infectivity of bare viral RNA when it was announced by Berkeley and Tübingen scientists (110, 111). Watson claimed to have played a joke on Robley Williams, who was presenting the TMV RNA results at the Ciba Symposium, by forging a telegram from Stanley conveying the breaking news that TMV protein alone was infectious (112, p. 217).

The infectivity of viral RNA overturned the initial interpretation of the reconstitution experiment, the creation of functional virus out of inert components. In any event, the genetic role of RNA—which the reconstituted TMV hybrids made undeniable—was more consequential. Stanley emphasized that (as he put it in 1956) "the ribonucleic acid core of the virus carries the genetic message" (113, p. 816). Crick regarded the hybrid reconstitution experiment as providing "the most convincing evidence that RNA is responsible for the specific construction of proteins" (114, p. 198). Part of what was attractive about TMV as an RNA virus is that it seemed to provide direct access to messenger RNA (mRNA). George Gamow, Alex Rich, and Martynas Yčas were already trying to use correlations between RNA mutations and CP composition of different TMV and turnip yellow mosaic virus (TYMV) strains to crack the genetic code. Unfortunately, as they explained, "the data for TMV and TY[M]V lead to different and contradictory assignments between the amino acids and the base triplets" (115, p. 56). As Stanley had observed in reading their draft paper, one could not necessarily assume the Chargaff rule for RNA (116). But with both the RNA and protein chemically available, TMV seemed the best system to elucidate their coding relation. Once the complete protein sequence was in hand, Fraenkel-Conrat optimistically claimed TMV would become "the Rosetta stone of biochemical genetics, in supplying the bilingual record needed to decipher nucleic acid in terms of protein structure" (117, p. 810).

It took until 1960 to completely sequence the TMV CP (118, 119). By that time, chemical methods for mutagenizing nucleic acids provided a powerful new tool for correlating changes in TMV RNA with those in protein sequence. The Tübingen group took the lead on this, generating mutants of TMV with nitrous acid (58, 120, 121). As Karl Mundry recalled, the amino acid analyzers in Tübingen were running "day and night" to detect any TMV protein variants, which they did find (122, p. 158). The German data showed that not all nucleic acid mutations showed up as changes in the CP (123). Fraenkel-Conrat obtained the nitrous acid incubation protocol from Mundry, and the Berkeley group soon produced its own mutant with three amino acid substitutions (124). But they realized that trying to crack the genetic code this way with TMV, mutant by mutant, was like "climbing the Mt. Everest of molecular biology" (125, p. 144). As it turned out, the 1961 development of a cell-free translation system by Marshall Nirenberg and Heinrich Matthaei provided a much swifter way up the mountain (126). For a time, Nirenberg and Matthaei used purified TMV nucleic acid (among other sources) as a template for work on cell-free translation, before synthetic RNAs became the preferred material (127, 128).

THE LAST HALF CENTURY

Although TMV was sidelined in the race to crack the genetic code, it remained an important system for molecular biology and plant science. To do justice to the post-1965 era would require another review, but a few highlights illustrate its continuing value. In 1968 and 1969, Itaru Takere and Yoshiarki Otsuki published their field-transforming method for preparing plant cell protoplasts for TMV infection (129, 130). This enabled single-step, synchronous virus growth in the

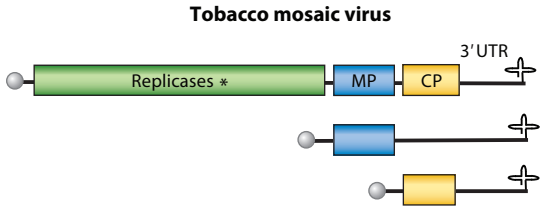

Figure 6

Genomic composition and expression of tobacco mosaic virus (TMV) RNA. Wild-type TMV transcripts consist of a 5′ cap structure (*gray beads*) and a 3′ transfer RNA-like structure adjacent to the 3′ untranslated region (UTR). TMV encodes two replicase-associated proteins (replicases), with the longer being translated by means of a ribosomal read-through amber stop codon (denoted by *asterisk*). Two subgenomic messenger RNAs are produced during replication: The longer is used for translation of the cell-to-cell movement protein (MP), and the second is for the coat protein (CP). Figure adapted with permission from original figure by Herman B. Scholthof.

host, an advantage phage workers had enjoyed for 30 years (45). Work on in vitro assembly of TMV as well as on its atomic structure proceeded apace. Aaron Klug became the head of the virus research group Franklin had assembled, which moved in 1962 into the Laboratory of Molecular Biology in Cambridge. In 1982, Klug received the Nobel Prize in Chemistry in recognition of the work he began with Franklin. The Cambridge group and others developed detailed models for the self-assembly of TMV from its CP (involving 34 subunit disks) and RNA (131–134). By 1989, the atomic structure of TMV had been refined to 2.9 Å by Gerald Stubbs's laboratory (135).

On the genetic side, the mRNA for the CP of TMV was identified in 1976 (136). TMV's ~6,400-bp RNA sequence was published in 1982; it was one of the first complete viral genomes published (137). By that time, plant virologists were studying the gene products besides CP; TMV was the first virus shown to encode a movement protein (MP) (138, 139) (**Figure 6**). MP increases the permeability of plasmodesmata to facilitate cell-to-cell movement of TMV, binding to RNA and interacting with cytoskeletal elements (140–142). The first transgenic plants were created using TMV in Roger Beachy's laboratory, demonstrating the concept of CP-mediated cross protection (143). This method of host protection made its way into commercial application, closing the circle from the discovery of TMV in the field more than a century earlier.

In sum, TMV has contributed in countless ways to basic knowledge about viruses, molecular genetics, and the dynamics of infection. Its history as a research object also tells us something about the nature of modern life science and how breakthroughs are reported to the public. Experiments that make a big splash are often presented by journalists in ways that do not capture their lasting significance. What was important about TMV's crystallization was less the feat of precipitation than the effectiveness of a chemical approach. In 1955, headlines about creating life in a test tube with TMV protein and nucleic acid quickly became dated. Now we see the reconstitution experiment as illuminating the nature of viral self-assembly and the genetic role of RNA. Less visible in media reports were the myriad ways that TMV provided a model for studying human pathogens such as influenza and polio (5, 144). In our pandemic age of renewed public interest in viruses, TMV's history reminds us of the enduring—and often unanticipated—value of basic science on model systems.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The author thanks Daniel DiMaio, Yaacob Dweck, Gregory Morgan, Karen-Beth Scholthof, and Herman Scholthof for comments on an early draft of this review and for help with figures.

LITERATURE CITED

1. Ankeny RA, Leonelli S. 2020. *Model Organisms*. New York: Cambridge Univ. Press
2. Dietrich MR, Ankeny RA, Crowe N, Green S, Leonelli S. 2020. How to choose your research organism. *Stud. Hist. Philos. Sci. Part C: Stud. Hist. Philos. Biol. Biomed. Sci.* 80:101227
3. Delbrück M, ed. 1950. *Viruses 1950. Proceedings of a Conference on the Similarities and Dissimilarities Between Viruses Attacking Animals, Plants, and Bacteria, Respectively*. Pasadena, CA: Div. Biol., Calif. Inst. Technol.
4. Cairns J, Stent GS, Watson JD, eds. 1966. *Phage and the Origins of Molecular Biology*. Cold Spring Harbor, NY: Cold Spring Harb. Lab.
5. Creager ANH. 2002. *The Life of a Virus: Tobacco Mosaic Virus as an Experimental Model, 1930–1965*. Chicago: Univ. Chicago Press
6. Tushingham S, Snyder CM, Brownstein KJ, Damitio WJ, Gang DR. 2018. Biomolecular archaeology reveals ancient origins of indigenous tobacco smoking in North American Plateau. *PNAS* 115:11742–47
7. van Helvoort T. 1991. What is a virus? The case of tobacco mosaic disease. *Stud. Hist. Philos. Sci. Part A* 22:557–88
8. Mayer A. 1886. Über die Mosaikkrankheit des Tabaks. *Landwirtsch. Vers.-Stationen* 32:451–67
9. Ivanovskii D. 1892. Über die Mosaikkrankheit der Tabakspflanze. *Bull. L'Acad. Imp. Sci. St. Pétersbourg* 35:67–70
10. Beijerinck MW. 1898. Über ein Contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblätter. *Verh. K. Akad. Weterschappen Amst. Afd. Natuurkunde* 6:3–21
11. Sankaran N. 2018. On the historical significance of Beijerinck and his *contagium vivum fluidum* for modern biology. *Hist. Philos. Life Sci.* 40:41
12. Levine AJ. 1996. The origins of virology. In *Fields Virology*, ed. BN Fields, DM Knipe, PM Howley, pp. 1–14. Philadelphia, PA: Lippincott-Raven. 3rd ed.
13. Hughes SS. 1977. *The Virus: A History of the Concept*. New York: Sci. Hist. Publ.
14. Loeffler F, Frosch P. 1898. Berichte der Kommission zur Erforschung der Maul- und Klauenseuche bei dem Institut für Infektionskrankheiten in Berlin. *Z. Bakteriol. Parasitenkd. Infekt. Abt. I* 23:371–91
15. Sanarelli G. 1898. Das myxomatogene Virus: Beitrag zum Studium der Krankheitserreger ausserhalb des Sichtbaren. *Z. Bakteriol. Parasitenkd. Infekt. Abt. I* 23:865–73
16. M'Fadyean J. 1900. African horse-sickness. *J. Comp. Pathol. Ther.* 13:1–20
17. Lode A, Gruber J. 1901. Bakteriologische Studien über die Ätiologie einer epidemischen Erkrankung der Hühner in Tirol 1901. *Z. Bakteriol. Parasitenkd. Infekt. Abt. I* 30:593–604
18. Centanni E. 1902. Die Vogelpest: Beitrag zu dem durch Kerzen filtrierbaren Virus. *Z. Bakteriol. Parasitenkd. Infekt. Abt. I* 31:145–52, 182–201
19. Maggioria A, Valenti GL. 1903. Über eine Seuche von exsuditivem Typhus bei Hühnern. *Z. Hyg. Infektionskr.* 42:185–243
20. Reed W, Carroll J. 1902. The etiology of yellow fever (a supplemental note). *Am. Med.* 3:980
21. Olitsky PK. 1924. Experiments on the cultivation of the active agent of mosaic disease of tobacco and tomato. *Science* 60:593–94
22. Creager ANH, Scholthof KB, Citovsky V, Scholthof HB. 1999. Tobacco mosaic virus. Pioneering research for a century. *Plant Cell* 11:301–8
23. Scholthof K-BG. 2004. Tobacco mosaic virus: a model system for plant biology. *Annu. Rev. Phytopathol.* 42:13–34
24. Holmes FO. 1928. Accuracy in quantitative work with tobacco mosaic virus. *Bot. Gaz.* 86:66–81
25. Holmes FO. 1929. Local lesions in tobacco mosaic. *Bot. Gaz.* 87:39–55
26. Scholthof K-BG. 2014. Making a virus visible: Francis O. Holmes and a biological assay for *Tobacco mosaic virus*. *J. Hist. Biol.* 47:107–45
27. Purdy HA. 1929. Immunologic reactions with tobacco mosaic virus. *J. Exp. Med.* 49:919–35

28. Scholthof KG, Peterson PD. 2006. The role of Helen Purdy Beale in the early development of plant serology and virology. *Adv Appl. Microbiol.* 59:221–41
29. Vinson CG. 1927. Precipitation of the virus of tobacco mosaic. *Science* 66:357–58
30. Vinson CG, Petre AW. 1931. Mosaic disease of tobacco, part 2. Activity of the virus precipitated by lead acetate. *Contrib. Boyce Thompson Inst.* 3:131–45
31. Northrop JH. 1930. Crystalline pepsin. Part I. Isolation and tests of purity. *J. Gen. Physiol.* 13:739–66
32. Northrop JH, Kunitz M. 1932. Crystalline trypsin. Part I. Isolation and tests of purity. *J. Gen. Physiol.* 16:267–94
33. Stanley WM. 1935. Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus. *Science* 81:644–45
34. Kay LE. 1986. W.M. Stanley's crystallization of the tobacco mosaic virus, 1930–1940. *Isis* 77:450–72
35. Crystals isolated at Princeton believed unseen disease virus. 1935. *New York Times*, June 28, p. 1
36. Bawden FC, Pirie NW, Bernal JD, Fankuchen I. 1936. Liquid crystalline substances from virus-infected plants. *Nature* 138:1051–52
37. Bawden FC, Pirie NW. 1937. The isolation and some properties of liquid crystalline substances from solanaceous plants infected with three strains of tobacco mosaic virus. *Proc. R. Soc. Lond. B* 123:274–320
38. Stanley WM, Loring HS. 1936. The isolation of crystalline tobacco mosaic virus protein from diseased tomato plants. *Science* 83:85
39. Stanley WM, Wyckoff RWG. 1937. The isolation of tobacco ring spot and other virus proteins by ultracentrifugation. *Science* 85:181–83
40. Stanley WM. 1939. The isolation and properties of tobacco ring spot virus. *J. Biol. Chem.* 129:405–28
41. Stanley WM. 1940. Purification of tomato bushy stunt virus by differential centrifugation. *J. Biol. Chem.* 135:437–54
42. Stent GS. 1966. Introduction: waiting for the paradox. In *Phage and the Origins of Molecular Biology*, ed. J Cairns, GS Stent, JD Watson, pp. 3–8. Cold Spring Harbor, NY: Cold Spring Harb. Lab.
43. Muller HJ. 1936. Physics in the attack on the fundamental problems of genetics. *Sci. Mon.* 44:210–14
44. Delbrück M. 1970. A physicist's renewed look at biology: twenty years later. *Science* 168:1312–15
45. Ellis EL, Delbrück M. 1939. The growth of bacteriophage. *J. Gen. Physiol.* 22:365–84
46. Wyckoff RWG, Corey RB. 1936. The ultracentrifugal crystallization of tobacco mosaic virus protein. *Science* 84:513
47. Viruses broken up by centrifugal forces. 1937. *New York Times*, June 24, p. 27
48. Price WC, Wyckoff RWG. 1939. Ultracentrifugation of juices from plants affected by tobacco necrosis. *Phytopathology* 29:83–94
49. Beard JW, Wyckoff RWG. 1937. The isolation of a homogeneous heavy protein from virus-induced rabbit papillomas. *Science* 85:201–2
50. Wyckoff RWG. 1937. Ultracentrifugal study of virus proteins. *Proc. Am. Philos. Soc.* 77:455–62
51. Fruton JS. 1972. *Molecules and Life: Historical Essays on the Interplay of Chemistry and Biology*. New York: Wiley-Interscience
52. Stanley WM. 1938. *Letter to Bawden, 19 Jan 1938*. Letter, Wendell M. Stanley Papers, 78/18c, carton 1, folder January 1938, Bancroft Library, Univ. Calif. Berkeley
53. Kausche GA, Pfannkuch E, Ruska H. 1939. Die Sichtbarmachung von pflanzlichem Virus im Übermikroskop. *Naturwissenschaften* 27:292–98
54. Stanley WM, Anderson TF. 1941. A study of purified viruses with the electron microscope. *J. Biol. Chem.* 139:325–38
55. Bernal JD, Fankuchen I. 1941. X-ray and crystallographic studies of plant virus preparations: I. Introduction and preparation of specimens II. Modes of aggregation of the virus particles. *J. Gen. Physiol.* 25:111–46
56. Butenandt A. 1977. The historical development of modern virus research in Germany, especially in the Kaiser-Wilhelm-/Max-Planck-Society, 1936–1954. *Med. Microbiol. Immunol.* 164:3–14
57. Rheinberger H-J. 2000. Virusforschung an den Kaiser-Wilhelm-Instituten für Biochemie und für Biologie. In *Geschichte der Kaiser-Wilhelm-Gesellschaft im Nationalsozialismus Bd. 2*, ed. D Kaufmann, pp. 667–98. Göttingen, Ger.: Wallstein

58. Brandt C. 2004. *Metapher und Experiment: Von der Virusforschung zum genetischen Code*. Göttingen, Ger.: Wallstein
59. Lewis J. 2004. Virus research to molecular biology: tobacco mosaic virus in Germany, 1936–1956. *J. Hist. Biol.* 37:259–301
60. Melchers G. 1938. *Letter to Stanley, 23 Mar 1938*. Letter, Wendell M. Stanley Papers, 78/18c, carton 10, folder Melchers, Johann George Friedrich, Bancroft Library, Univ. Calif. Berkeley
61. Schramm G. 1943. Über die Spaltung des Tabakmosaikvirus in niedermolekulare Proteine und die Rückbildung hochmolekularen Proteins aus den Spaltstücken. *Die Naturwissenschaften* 31:94–96
62. Schramm G. 1947. Über die Spaltung des Tabakmosaikvirus und die Wiedervereinigung der Spaltstücke zu höhermolekularen Proteinen. II. Versuche zur Wiedervereinigung der Spaltstücke. *Z. Naturforsch.* 2B:249–57
63. Watson JD. 1968. *The Double Helix: A Personal Account of the Discovery of the Structure of DNA*. New York: Atheneum
64. Deichmann U. 1996. *Biologists Under Hitler*. Cambridge, MA: Harvard Univ. Press
65. Harris JI, Li CH. 1952. The biological activity of enzymatic digests of insulin. *J. Am. Chem. Soc.* 74:2945–46
66. Fraenkel-Conrat H. 1979. Protein chemists encounter viruses. *Ann. N.Y. Acad. Sci.* 325:309–15
67. Harris JI, Knight CA. 1952. Action of carboxypeptidase on tobacco mosaic virus. *Nature* 170:613–14
68. Fraenkel-Conrat H. 1986. The history of tobacco mosaic virus and the evolution of molecular biology. In *The Plant Viruses*, ed. MHV van Regenmortel, H Fraenkel-Conrat, pp. 5–17. New York: Plenum
69. Schramm G, Braunitzer G, Schneider JW. 1954. Zur Bestimmung der Amino-Endgruppe im Tabakmosaikvirus. *Z. Naturforsch.* 9B:298–301
70. Niu C-I, Fraenkel-Conrat H. 1955. C-terminal amino-acid sequence of tobacco mosaic virus protein. *Biochim. Biophys. Acta* 16:597–98
71. Schramm G. 1954. Die Struktur des Tabakmosaikvirus und seiner Mutanten. *Adv. Enzymol.* 15:449–84
72. Ryle AP, Sanger F, Smith LF, Kitai R. 1955. The disulphide bonds of insulin. *Biochem. J.* 60:541–56
73. de Chadarevian S. 1996. Sequences, conformation, information: biochemists and molecular biologists in the 1950s. *J. Hist. Biol.* 29:361–86
74. Fraenkel-Conrat H. 1954. Reaction of nucleic acid with formaldehyde. *Biochim. Biophys. Acta* 15:307–9
75. Hershey AD, Chase M. 1952. Independent functions of viral protein and nucleic acid in growth of bacteriophage. *J. Gen. Physiol.* 36:89–96
76. Knight CA. 1954. The chemical constitution of viruses. *Adv. Virus Res.* 2:153–82
77. Olby RC. 2009. *Francis Crick: Hunter of Life's Secrets*. Cold Spring Harbor, NY: Cold Spring Harb. Lab.
78. Creager ANH, Morgan GJ. 2008. After the double helix: Rosalind Franklin's research on *Tobacco mosaic virus*. *Isis* 99:239–72
79. Watson JD. 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
80. Watson JD, Crick FHC. 1953. A structure for deoxyribose nucleic acid. *Nature* 171:737–38
81. Franklin RE, Gosling RG. 1953. Molecular configuration in sodium thymonucleate. *Nature* 171:740–41
82. Wilkins MHF, Stokes AR, Seeds WE, Oster G. 1950. Tobacco mosaic virus crystals and three-dimensional microscopic vision. *Nature* 166(4212):127–29
83. Franklin R. 1954. *Letter to Stanley, 7 May 1954*. Letter, Wendell M. Stanley Papers, carton 8, folder-Franklin, Rosalind, 78/18c, Bancroft Library, Univ. Calif. Berkeley
84. Franklin RE. 1954. *Letter to Stanley, 6 Jul 1954*. Letter, Wendell M. Stanley Papers, carton 8, folder Franklin, Rosalind, 78/18c, Bancroft Library, Univ. Calif. Berkeley
85. Franklin RE. 1954. *Letter to Stanley, 14 Oct 1954*. Letter, Wendell M. Stanley Papers, carton 8, folder Franklin, Rosalind, 78/18c, Bancroft Library, Univ. Calif. Berkeley
86. Franklin RE. 1956. *Letter to Stanley, 4 Jul 1956*. Letter, Wendell M. Stanley Papers, carton 8, folder Franklin, Rosalind, 78/18c, Bancroft Library, Univ. Calif. Berkeley
87. Franklin RE, Commoner B. 1955. Abnormal protein associated with tobacco mosaic virus: X-ray diffraction by an abnormal protein (B8) associated with tobacco mosaic virus. *Nature* 175:1077–82
88. Watson JD. 1954. *Letter to Crick, 15 Oct 1954*. Letter, Francis Crick Papers, National Library of Medicine, Profiles in Science. **https://collections.nlm.nih.gov/catalog/nlm:nlmuid-101584582X121-doc**

89. Rich A, Watson JD. 1954. Some relations between DNA and RNA. *PNAS* 40:759–64
90. Watson JD. 1954. *Letter to Delbrück, 25 Mar 1954*. Letter, Max Delbrück Papers, California Institute of Technology Archives, box 23, folder 23
91. Rich A, Dunitz JD, Newmark P. 1955. Abnormal protein associated with tobacco mosaic virus: structure of polymerized tobacco plant protein and tobacco mosaic virus. *Nature* 175(4468):1074–75
92. Franklin RE. 1955. Structure of tobacco mosaic virus. *Nature* 175:379–81
93. Caspar DLD. 1956. Structure of tobacco mosaic virus: radial density distribution in the tobacco mosaic virus particle. *Nature* 177(4516):928
94. Franklin RE. 1956. Structure of tobacco mosaic virus: location of the ribonucleic acid in the tobacco mosaic virus particle. *Nature* 177:928–30
95. Crick FHC, Watson JD. 1956. Structure of small viruses. *Nature* 177:473–75
96. Morgan GJ. 2003. Historical review: viruses, crystals and geodesic domes. *Trends Biochem. Sci.* 28:86–90
97. Morgan GJ. 2004. Early theories of virus structure. In *Conformational Proteomics of Macromolecular Architecture: Approaching the Structure of Large Molecular Assemblies and Their Mechanisms of Action*, ed. RH Cheng, L Hammar, pp. 3–40. Singapore: World Sci. Publ.
98. Crick FHC, Watson JD. 1957. Virus structure: general principles. See Ref. 111, pp. 5–13
99. Franklin RE, Klug A, Holmes KC. 1957. X-ray diffraction studies of the structure and morphology of tobacco mosaic virus. See Ref. 111, pp. 39–52
100. Fraenkel-Conrat H, Williams RC. 1955. Reconstitution of active tobacco mosaic virus from its inactive protein and nucleic acid components. *PNAS* 41:690–98
101. Scientists create life in test tube. 1955. *Pittsburgh Press*, Oct. 28, p. 1
102. Kaempffert W. 1955. Reconstruction of virus in laboratory reopens the question: What is life? *New York Times*, Oct. 30, p. E9
103. Silverman M. 1956. Now—MAN-MADE VIRUS—First step in controlling heredity? *Collier's*, Aug. 31, pp. 74–77
104. Fraenkel-Conrat H. 1956. The role of the nucleic acid in the reconstitution of active tobacco mosaic virus. *J. Am. Chem. Soc.* 78:882–83
105. Fraenkel-Conrat H, Singer B. 1957. Virus reconstitution. II. Combination of protein and nucleic acid from different strains. *Biochim. Biophys. Acta* 24:540–48
106. Gierer A, Schramm G. 1956. Infectivity of ribonucleic acid from tobacco mosaic virus. *Nature* 177:702–3
107. Gierer A. 1957. Structure and biological function of ribonucleic acid from tobacco mosaic virus. *Nature* 179:1297–99
108. Cohen S. 2008. Stanley, Wendell Meredith. In *Complete Dictionary of Scientific Biography*, ed. C Coulston Gillispie, FL Holmes, N Koertgeed, pp. 841–48. Detroit, MI: Charles Scribner's Sons
109. Harrison BD. 1999. The chemical nature of tobacco mosaic virus particles. In *Tobacco Mosaic Virus: One Hundred Years of Contributions to Virology*, ed. K-BG Scholthof, JG Shaw, M Zaitlin, pp. 71–73. St. Paul, MN: APS
110. Alfrey VG. 1957. *Cellular Biology, Nucleic Acids and Viruses*, Vol. 5. New York: NY Acad. Sci.
111. Wolstenholme GEW, Millar ECP, eds. 1957. *Ciba Foundation Symposium on the Nature of Viruses*. London: J & A Churchill
112. Watson JD. 2002. *Genes, Girls and Gamow*. New York: Alfred A. Knopf
113. Stanley WM. 1956. Virus composition and structure; 25 years ago and now. *Fed. Proc.* 15:812–18
114. Crick F. 1957. Nucleic acids. *Sci. Am.* 197:188–200
115. Gamow G, Rich A, Yčas M. 1956. The problem of information transfer from the nucleic acids to proteins. *Adv. Biol. Med. Phys.* 4:23–68
116. Stanley WM. 1955. *Letter to Gamow, 1 Apr 1955*. Letter, Wendell M. Stanley Papers, 78/18c, carton 8, folder Gamov, George, Bancroft Library, Univ. Calif. Berkeley
117. Fraenkel-Conrat H. 1957. Degradation and structure of tobacco mosaic virus. *Fed. Proc.* 16:810–15
118. Anderer FA, Uhlig H, Weber E, Schramm G. 1960. Primary structure of the protein of tobacco mosaic virus. *Nature* 186:922–25
119. Tsugita A, Gish DT, Young J, Fraenkel-Conrat H, Knight CA, Stanley WM. 1960. The complete amino acid sequence of the protein of tobacco mosaic virus. *PNAS* 46:1463–69

120. Gierer A, Mundry K-W. 1958. Production of mutants of tobacco mosaic virus by chemical alteration of its ribonucleic acid *in vitro*. *Nature* 182(4647):1457–58
121. Mundry KW, Gierer A. 1958. Die Erzeugung von Mutationen des Tabakmosaikvirus durch chemische Behandlung seiner Nucleinsäure *in vitro*. *Z. Vererbungslehre* 89(4):614–30
122. Mundry KW. 1999. TMV in Tübingen (1945–1965). In *Tobacco Mosaic Virus: One Hundred Years of Contributions to Virology*, ed. K-BG Scholthof, JG Shaw, M Zaitlin, pp. 155–160. St. Paul, MN: APS
123. Wittmann HG. 1959. Vergleich der Proteine des Normalstammes und einer Nitritmutante des Tabakmosaikvirus. *Z. Vererbungslehre* 90:463–75
124. Tsugita A, Fraenkel-Conrat H. 1960. The amino acid composition and C-terminal sequence of a chemically evoked mutant of TMV. *PNAS* 46:636–42
125. Fraenkel-Conrat H, Stanley WM. 1961. The chemistry of life. Part 2. Implications of recent studies of a simple virus. *Chem. Eng. News* 39:136–44
126. Nirenberg MW, Matthaei JH. 1961. The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polyribonucleotides. *PNAS* 47:1588–602
127. Tsugita A, Fraenkel-Conrat H, Nirenberg MW, Matthaei JH. 1962. Demonstration of the messenger role of viral RNA. *PNAS* 48:846–53
128. Kay LE. 2000. *Who Wrote the Book of Life? A History of the Genetic Code*. Stanford, CA: Stanford Univ. Press
129. Takebe I, Otsuki Y, Aoki S. 1968. Isolation of tobacco mesophyll cells in intact and active state. *Plant Cell Physiol.* 9:115–24
130. Takebe I, Otsuki Y. 1969. Infection of tobacco mesophyll protoplasts by tobacco mosaic virus. *PNAS* 64:843–48
131. Butler PJ, Klug A. 1971. Assembly of the particle of tobacco mosaic virus from RNA and disks of protein. *Nat. New Biol.* 229:47–50
132. Lebeurier G, Nicolaieff A, Richards KE. 1977. Inside-out model for self-assembly of tobacco mosaic virus. *PNAS* 74:149–53
133. Butler PJ, Finch JT, Zimmern D. 1977. Configuration of tobacco mosaic virus, RNA during virus assembly. *Nature* 265:217–19
134. Butler PJ. 1984. The current picture of the structure and assembly of tobacco mosaic virus. *J. Gen. Virol.* 65(2):253–79
135. Namba K, Pattanayek R, Stubbs G. 1989. Visualization of protein-nucleic acid interactions in a virus: refined structure of intact tobacco mosaic virus at 2.9 Å resolution by X-ray fiber diffraction. *J. Mol. Biol.* 208:307–25
136. Hunter TR, Hunt T, Knowland J, Zimmern D. 1976. Messenger RNA for the coat protein of tobacco mosaic virus. *Nature* 260:759–64
137. Goelet P, Lomonossoff GP, Butler PJ, Akam ME, Gait MJ, Karn J. 1982. Nucleotide sequence of tobacco mosaic virus RNA. *PNAS* 79:5818–22
138. Nishiguchi M, Motoyoshi F, Oshima N. 1978. Behaviour of a temperature sensitive strain of tobacco mosaic virus in tomato leaves and protoplasts. *J. Gen. Virol.* 39:53–61
139. Deom CM, Oliver MJ, Beachy RN. 1987. The 30-kilodalton gene product of tobacco mosaic virus potentiates virus movement. *Science* 237:389–94
140. Citovsky V, Knorr D, Schuster G, Zambryski P. 1990. The P30 movement protein of tobacco mosaic virus is a single-strand nucleic acid binding protein. *Cell* 60:637–47
141. McLean BG, Zupan J, Zambryski PC. 1995. Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. *Plant Cell* 7:2101–14
142. Wolf S, Deom CM, Beachy RN, Lucas WJ. 1989. Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. *Science* 246:377–79
143. Abel PP, Nelson RS, De B, Hoffmann N, Rogers SG, et al. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* 232:738–43
144. Creager ANH. 2016. Paradigms and exemplars meet biomedicine. In *Kuhn's Structure of Scientific Revolutions at Fifty: Reflections on a Science Classic*, ed. RJ Richards, L Daston, pp. 151–66. Chicago: Univ. Chicago Press
145. Fraenkel-Conrat H. 1956. Rebuilding a virus. *Sci. Am.* 194(6):42–47