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Coat-protein-mediated resistance to tobacco mosaic virus: discovery mechanisms and exploitation

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In 1986 we reported that transgenic plants which accumulate the coat protein of tobacco mosaic virus (TMV) are protected from infection by TMV, and by closely related tobamoviruses. The phenomenon is referred to as coat-protein-mediated resistance (CP-MR), and bears certain similarities to cross protection, a phenomenon described by plant pathologists early in this century. Our studies of CP-MR against TMV have demonstrated that transgenically expressed CP interferes with disassembly of TMV particles in the inoculated transgenic cell. However, there is little resistance to local, cell-to-cell spread of infection. CP-MR involves interaction between the transgenic CP and the CP of the challenge virus, and resistance to TMV is greater than to tobamo viruses that have CP genes more distantly related to the transgene. Using the known coordinates of the three-dimensional structure of TMV we developed mutant forms of CP that have stronger inter-subunit interactions, and confer increased levels of CP-MR compared with wild-type CP. Similarly, it is predicted that understanding the cellular and structural basis of CP-MR will lead to the development of variant CP transgenes that each can confer high levels of resistance against a range of tobamoviruses.

Keywords: tobacco mosaic virus; coat-protein-mediated resistance; subunit structure

1. EARLY DEVELOPMENTS IN GENE EXPRESSION AND PLANT TRANSFORMATION

During the 1970s and 1980s there was a great deal of interest in understanding the nature of genes and the DNA sequences that control their expression. While the vast majority of the research involved studies in yeasts and other simple eukaryotic organisms, a number of research groups used plants as study systems and obtained enough data to demonstrate that plant gene expression was not significantly different from that in other organisms. At this time, several research groups were studying plant genes involved in light-regulated and tissue-specific gene expression in the hope that they would identify the rules of gene regulation and plant development, and would learn how plants respond to changes in the biotic and abiotic environment.

Concurrently, methods were being developed to introduce foreign genes into yeast, animal, and plant cells, and to select stably transformed cell lines. Transformation of bacterial and eukaryotic cells was accomplished by the mid-1970s, and the first transgenic plants were developed via *Agrobacterium*-mediated transformation in 1983. This event was widely heralded as marking the beginning of a science that would result in novel crops and improved techniques for plant breeding. In anticipation of successful genetic transformation, other research groups, including ours, were developing genes that could, it was hoped, later be used to produce transgenic plants possessing useful new characteristics.

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It soon became clear that the standard gene expression cassettes that worked well in yeasts (e.g. animal promoters of genes in metabolic pathways) or in animal cells (e.g. based on promoters from SV40, or other animal viruses) would not be useful for gene expression in plant cells. Several research groups, including those of plant virologists Robert Shepherd (University of California, Davis, USA) and Ken Richards (Strasbourg, France), searched for strong constitutive promoters that might be used to construct cassettes for gene expression in plants. Studies of the plant pararetrovirus, cauliflower mosaic virus (CaMV), led ultimately to the identification of promoters for the 19S and 35S transcripts of the virus (Guilley *et al.* 1982). The 19S promoter was originally thought to be the major promoter for CaMV; however, it was later shown that the 35S promoter was superior, and would lead to greater levels of gene expression in transgenic plants than the 19S promoter, or promoters from the T-DNA of *Agrobacterium tumefaciens* itself (Sanders *et al.* 1987).

In 1980 and 1981, we considered the possibility of developing transgenic plants that would express sequences derived from tobacco mosaic virus (TMV). One of the driving forces for our work was an interest in viral pathogenicity, and the desire to test the hypothesis that one or more virus genes might be capable of inducing disease. Work by A. Siegel, M. Zaitlin, S. Wildman and others between 1955 and 1975 had shown that infection of tobacco plants with TMV resulted in changes in photosynthesis, and had demonstrated the accumulation of pseudovirions in chloroplasts (Goodman *et al.* 1986). We found that in certain leaves, but not others, the coat protein (CP) was associated with thylakoid membranes

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and was probably causally associated with reduced electron transport (Reinero & Beachy 1989). To examine the possibility that CP would affect electron transport in the absence of TMV infection, we proposed to develop transgenic plants that produced CP.

A second and complementary reason to develop transgenic plants that expressed virus sequences was to address the following long-standing question: what is the basis of cross protection against severe strains of TMV in plants previously infected by a mild strain? The debate had already generated hypotheses stating that cross protection was the result of metabolic changes in the host, that RNA–RNA interactions interfered with virus replication, or that CP produced by the protecting strain interfered with infection by the challenge virus (Gibbs 1969; de Zoeten & Fulton 1975; Sherwood & Fulton 1982). When our studies were initiated, there were data to suggest that one or possibly more factors were responsible for cross protection. We and others (e.g. Hamilton 1980) proposed that it would be possible to develop plants that were 'genetically engineered for cross protection' if one could identify a transgene that encoded the protein or RNA that conferred protection. Eventually, we constructed transgenic plants that produced either TMV CP, 30 kDa movement protein, non-translated CP RNA sequences or antisense gene sequences. Each of these types of plant were to be tested for 'cross protection' against TMV infection. Only the work with the CP will be discussed here as this proved to be the most interesting to us.

2. EARLY ATTEMPTS TO DESIGN THE CP GENE AND TESTS OF TRANSGENIC PLANTS

In the fall of 1981, I made a formal proposal to scientists and management at Monsanto Company (St Louis, MO, USA) to develop transgenic plants that contain TMV gene sequences, with major emphasis to be placed on expression of the CP gene, for the purpose of determining whether or not such plants would be protected against infection by TMV and other tobamoviruses. Following a period of discussions, relating to feasibility, with Robert T. Fraley and Steven G. Rogers, facilitated by Ernest Jaworski, we agreed to cooperate to develop transgenic plants. In my laboratory at Washington University, St Louis, we would develop the cloned cDNAs, and collaborate with Monsanto scientists to develop the corresponding chimeric genes. Transgenic plants would be prepared by the plant transformation group at Monsanto, and transgenic plants would be characterized and tested for virus resistance at Washington University. Early in 1982, Barun De, a post-doctoral associate, began by attempting to polyadenylate TMV-RNA to be used for standard cDNA cloning of the CP gene. Earlier work in Milton Zaitlin's laboratory had shown the location of the CP gene to be proximal to the 3′-end of the RNA (Beachy *et al*. 1976; Beachy & Zaitlin 1977). Unfortunately, these cDNA cloning attempts failed, probably because of inconsistent quality control of certain enzymes used for the procedures.

During the summer of 1982, I visited the John Innes Institute, Norwich, UK, and in discussions with George Lomonosoff learned that the sequence of the U1 strain of TMV had been determined. He generously gave me a copy of the manuscript that was subsequently published (Goelet *et al*. 1982) and, upon my return to the USA, we began in earnest to attempt to clone the CP coding sequence. Following a prolonged period of frustration that involved misconstruction of primer sets, poor ligation reactions and sequencing problems, Patricia Powell, a graduate student, and post-doctoral associate Barun De succeeded in isolating and sequencing the CP sequence. The gene sequence was ligated to to the 19S CaMV promoter by Steve Rogers, Robert Fraley, and their colleague Patricia Sanders. Co-integration of the intermediate plasmid with the disarmed Ti plasmid in *A. tumefaciens*, and finally, plant transformation were conducted by the Monsanto team (which included Nancy Hoffmann, who developed the transgenic plants). We elected to use *Nicotiana tabacum* cv Xanthi nn for these studies so that we could study resistance as a systemic, rather than a local lesion, reaction. Owing to the state of the art during the early 1980s, each of these procedures took much more time than anticipated. Our excitement at receiving the transgenic plantlets, and growing them to maturity led to disappointment when we characterized the plants in mid-1984. After confirming by Southern blot assays that the plants were transgenic, and that mRNA derived from the gene accumulated (albeit to very low levels) in some of the transgenic plant lines, we were unable to detect CP by Western blot assays. Unfortunately, the results were not changed by optimizing conditions for growth of the plants. These disappointing results led the research team to consider the possibility that the CP was unstable in the transgenic plants, or that the promoter was too weak for the purposes of these studies.

Our fortunes changed when colleagues at Monsanto Company found that the 35S promoter from CaMV caused significantly higher levels of transcription in transgenic plants than the 19S promoter. New intermediate plasmids were then constructed, and transgenic plants that carried the CP gene sequences with the 35S promoter were developed. It was with some trepidation that Patricia Powell carried out Western blot assays for CP, and there was great celebration when the results were positive. In the summer of 1985, we presented a short paper at a Symposium on Agricultural Biotechnology at Cornell University (Beachy *et al*. 1986) reporting the results of the studies to date. At that time, however, there were no data related to resistance or susceptibility to TMV of these plants. With the help of post-doctoral associate Richard Nelson, the parental R_0 plants were rooted and planted in soil, and after selecting plants of similar physical appearance, they were challenged by inoculation with TMV. As we monitored the plants (almost hourly!) during the next two weeks, and cautiously recorded the appearance of symptoms on the upper leaves, we came to the conclusion that plant lines that contained CP developed disease symptoms much later than cuttings of plants that did not contain CP. While this was an exciting result, the interpretation was tempered by the knowledge that plants regenerated from tissue culture may exhibit somaclonal variation. We agreed to wait to present our findings until the results of studies of R_1 progeny of transgenic plants could be evaluated for resistance. These experiments showed that R_1 progeny were also resistant to infection by TMV: plants

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that accumulated the CP (i.e. CP(+) plants) either escaped systemic infection or symptom development was delayed compared with CP(−) plants (see figure 1). However, when the concentration of TMV used for inoculation was increased, resistance was largely overcome. These findings were verified by several independent studies and the conclusions were judged as valid based upon statistical treatment of the data. The first public presentation of the data was made at the 1985 meeting of the American Crop Science Society, in Chicago, on December 5. While the results were exciting to some of us, and were reported in the public media, a single question was forthcoming from the floor of the conference hall at the conclusion of the presentation: 'Was there a yield penalty in virus resistant plants?' Not exactly an enthusiastic response to the first example of genetic engineering for disease resistance! Nevertheless, the results were reported in *Science* in May, 1986 (Powell *et al*. 1986), and are considered to be the first example of pathogen derived resistance (Sanford & Johnston 1985) in transgenic plants.

I sought advice from Milton Zaitlin as to what to call this type of resistance. He said, 'It's your discovery, you choose a name.' We started with 'genetically engineered cross protection'; however, when I used the phrase during a lecture at the Australian National University, Adrian Gibbs was quick to point out that as the mechanism(s) of cross protection was not known, the choice of wording was inappropriate. Soon thereafter the phenomenon was renamed 'coat-protein-mediated resistance' (i.e. CP-MR). While plant pathologists may disagree about whether or not 'tolerance' should be substituted for 'resistance', the terminology has stuck.

3. FIELD TRIALS OF COAT-PROTEIN-MEDIATED RESISTANCE IN TOMATO

Soon after resistance was confirmed in tobacco plants, Maude Hinchee and colleagues at Monsanto Company introduced the CP gene into tomato cells and regenerated transgenic plants. We were pleased when subsequent studies at Washington University showed that CP(+) tomato plants were resistant to relatively high concentrations of TMV. These plant lines were tested in the field near Jerseyville (Illinois, USA), in the summer of 1987. This first field test of a genetically engineered food crop, in full view of the press and with significant fanfare, went off without untoward incident. With each of the principal investigators taking part, the small group of transgenic plants were drill planted and then carefully tended by field hands, graduate students, post-doctorates, daughter K.C., and me through the next four months. We were extremely pleased to discover that the tomato plants were highly resistant to TMV and produced high yields of fruit not unlike the results achieved by classical cross protection (Nelson *et al*. 1987).

4. CHARACTERISTICS OF COAT-PROTEIN-MEDIATED RESISTANCE (CP-MR)

(a) *Resistance to local infection*

Following the report by Powell Abel *et al*. (1986) we conducted a series of studies using CP(+) transgenic *N. tabacum* cvs Xanthi nn and Xanthi NN to characterize the nature of resistance to TMV in plants that accumulated CP. Following inoculation with a range of concentrations of TMV, we found that CP(+) Xanthi NN plants produced between 0 and 5% of the numbers of necrotic local lesions (LL) that developed in CP(−) plants. However, like plants that are cross-protected by pre-infection with a mild strain of TMV (Sherwood & Fulton 1982), the resistant plants were more susceptible to infection by TMV-RNA than by TMV. Inoculation with TMV-RNA largely (but not totally) overcame resistance; CP(+) plants produced approximately 50% the number of LL that appeared on CP(−) plants in the same conditions (Nelson *et al*. 1987). However, in these experiments, the time at which the LL appeared, the phenotype of the lesions, and the rate of expansion of lesions was the same in CP(+) and CP(−) plants.

Figure 1. An early, clear demonstration of coat-protein-mediated resistance against TMV in transgenic plants. Each of the plants was inoculated with a severe yellow strain of TMV ten days prior to the photograph.

The results of studies with local lesion and systemic hosts suggested that CP(+) plants were resistant to infection but that, once infection was initiated, transgenic CP was ineffective against local spread of infection, and weakly effective against systemic spread (Wisniewski *et al*. 1990). Furthermore, as CP-MR is effective against infection by TMV, but not TMV-RNA, we proposed that transgenic CP blocks a step in infection that precedes release of viral RNA from the virion. We now suggest that the lack of resistance to local spread of infection (which occurs via plasmodesmata) reflects the fact that local cell-to-cell spread of infection does not involve CP (Dawson *et al*. 1988): we concluded that transgenic CP is poorly effective in restricting the spread of infection that does not involve virions.

(b) *CP-MR in transient assays*

To further investigate the impact of CP on early events in infection, we used protoplasts isolated from mesophyll cells of CP(+) and CP(−) leaves. Protoplasts were inoculated with TMV, TMV-RNA, or TMV that was pre-treated at high pH to partially strip off the CP, exposing the 5′-end of the viral RNA. While CP(+) protoplasts were very highly resistant to infection by TMV, they were much more susceptible to TMV-RNA and to partially stripped TMV (Register & Beachy 1988). These studies supported the hypothesis that CP blocked an early step in TMV infection.

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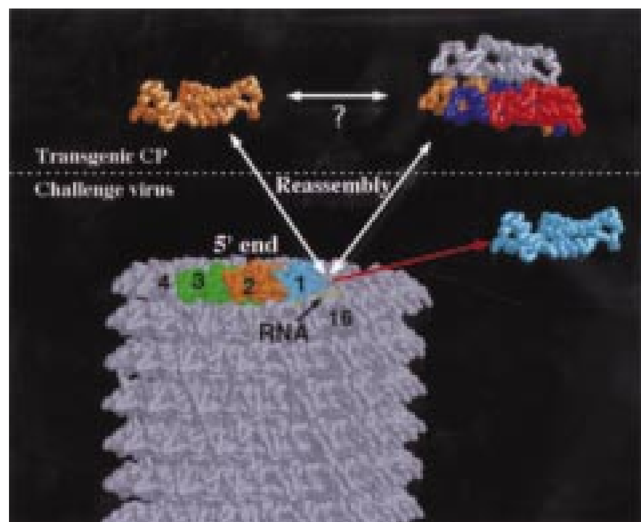

Figure 2. Computer-assisted graphic representation of a model that shows the predicted interactions between transgenic TMV CP (orange, upper) and challenge virus (grey, lower) that are involved in coat-protein-mediated resistance (from Bendahmane & Beachy 1998).

In a second study, we attempted to quantify the amount of CP required to confer resistance, and to characterize the nature of the CP that conferred CP-MR. Protoplasts were isolated from non-infected plants and different amounts of purified CP, or CP in different states of aggregation were introduced, along with TMV. Under these conditions, fewer cells that received the larger amounts of CP were infected than cells that received less CP. Furthermore, fewer of the cells that received aggregated CP (small aggregates to helical arrays of CP) were infected than of those that received monomers and small aggregates of CP. These studies may support the hypothesis that CP-MR results from binding by the CP, or virus-like particles, to an intracellular receptor, thereby preventing virus infection. Alternatively, such aggregates may stabilize the CP and serve as 'reservoirs' for monomers of CP that confer CP-MR. As discussed below, we now favour the hypothesis that monomers of CP in the transgenic cell interact in some manner with the challenge virus to prevent infection (Register & Beachy 1989).

These conclusions were supported by a study in transgenic plants conducted by Powell *et al.* (1989), in which it was shown that CP-MR occurred in plants that contained CP, but not in plants that accumulated untranslated mRNA from the gene. Furthermore, plants with greater levels of CP exhibited higher levels of CP-MR (see figure 2).

(c) *Early events in CP-MR*

In the mid-1980s it was shown by Michael Wilson that TMV undergoes cotranslational disassembly *in vitro*, and that this step was likely to be an early event in infection (Wilson & Perham 1985). These studies led to a collaborative project between John Shaw, M. Wilson and our laboratory using CP(+) plants. CP(+) and CP(−) protoplasts were inoculated with TMV particles that were double-labelled with ^{3}H-CP and ^{32}P-RNA, and distribution of the labels in Cs_2SO_4 gradients was examined at increasing times post-inoculation. In CP(−) protoplasts, ^{3}H-associated viral RNA was in the polyribosome fraction within 7 min post-infection and, by 60 min, most of the input viral RNA could not be recovered. In contrast, little or no virus was loaded with ribosomes in CP(+) protoplasts by 60 min (Wu *et al.* 1990). These studies supported the hypothesis that disassembly of TMV is restricted in CP(+) cells and that this is a major mechanism of CP-MR.

Concurrent with these studies Wilson and colleagues, using ISEM to immunotrap CP molecules, observed that there are pH 8-stable helical virus-like particles in extracts of CP(+) plants (Wilson 1989). The results of these studies raised several intriguing questions. What is the active form of CP? Does CP confer resistance as a single molecule, or as an aggregate of CP? Does the active CP bind to, and block a receptor in the cell at which TMV is disassembled, or does transgenic CP block disassembly by interacting directly with the challenge virus (Register *et al.* 1989)?

5. CP–CP INTERACTIONS IN CP-MR

(a) *Studies with related tobamoviruses suggest a role of CP sequences in CP-MR*

The results of the studies described above imply that the transgenic CP interacts in some manner with the challenge virus to prevent its disassembly, thereby restricting infection. Does transgenic CP interact with the CP or RNA of the challenge virus? We reasoned that if CP–CP interactions were important, resistance would be greater against tobamoviruses that have a high level of CP sequence identity with the transgenic CP than against viruses with lower levels of sequence identity. Furthermore, resistance would be unaffected by differences in viral RNA sequence. Nejidat & Beachy (1990) challenged CP(+) plants with a variety of tobamoviruses, including those closely related to TMV and those distantly related. This work demonstrated much greater levels of CP-MR against the viruses closely related to TMV than to the more distantly related viruses.

To determine the direct role of viral RNA, if any, in resistance, we constructed a chimeric virus comprising viral RNA that encoded TMV replicase and P30 movement protein, and CP of sunn hemp mosaic tobamovirus (SHMV). SHMV is very distantly related to TMV and CP(+) plants are very poorly resistant to challenge inoculation with SHMV (Nejidat & Beachy 1990). When CP(+) plants were challenged with the chimeric virus, the level of resistance was very low, and equivalent to resistance against SHMV (Clark *et al.* 1995). However, there were certain anomalies in this study, and definitive conclusions regarding the role of CP sequence similarities in CP-MR could not be drawn.

It was considered possible that resistance was caused by interaction of CP with the TMV or other tobamovirus origin of assembly sequence (OAS), and that this in some unknown manner, restricted virus replication. However, studies with the chimeric virus (Clark *et al.* 1995*b*) ruled out this possibility since the chimeric virus contained the TMV OAS, yet the level of resistance was very low. These experiments confirmed the conclusion that CP-MR was based upon similarities between the transgenic CP and the CP of the challenge virus, and not upon sequences in the challenge viral RNA.

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(b) *Structural mutants of TMV-CP and the role of CP–CP interactions in CP-MR*

To study the possible role(s) of protein structure and protein–protein interactions in CP-MR, we adopted a mutagenesis strategy, based upon the known structure of TMV and TMV CP, each of which are described in detail in other articles in this issue. In the first study, we developed transgenic plants that contained the PM2 mutant of TMV CP (Siegel *et al*. 1962). This CP, in which Thr28 was changed to Ile, assembles to form sheet-like aggregates, but does not assemble to form virus-like particles. Velocity sedimentation studies in sucrose density gradients showed that aggregates of CP-PM2 are relatively stable, and sediment at between 4S and 20S. Furthermore, transgenic plants producing CP-PM2 showed a high level of CP-MR against TMV. We interpreted these data to indicate that there was sufficient CP–CP interaction between CP-PM2 and the challenge virus to mediate resistance, but that the capacity to form virus-like particles was not required for CP-MR (Clark *et al*. 1995*a*). It should be mentioned that these results argue against the hypothesis that CP-MR is the result of binding of pseudovirions to a cellular receptor for TMV.

We then constructed a series of CP mutants by changing three highly conserved Thr residues, namely amino acids 28, 42, and 89, to Trp (i.e. creating CP mutants Thr28Trp, Thr42Trp, and Thr89Trp); in addition, a double mutant at positions 28 and 42 (Thr28Trp+ Thr42Trp) was constructed. Mutants of the CP were expressed by infectious cloned TMV cDNA as well as in transgenic plants. It was shown that mutant Thr28Trp was incapable of assembly, while the double mutant Thr28Trp+Thr42Trp and the mutant Thr89Trp produced protein aggregates of intermediate stability. Aggregates of mutant Thr42Trp CP were more stable than those of wild-type CP. When transgenic plants that contained mutant CP were challenged by inoculation with TMV, we observed a close correlation between the capacity of CP to assemble and CP-MR: CP-Thr28Trp did not confer resistance; CP-Thr28Trp+Thr42Trp, and CP-Thr89Trp conferred intermediate levels of resistance; and CP-Thr42Trp conferred very high levels of CP-MR, greater than those conferred by wild-type CP. The results were similar in local lesion or systemic hosts, and support the hypothesis that the degree of resistance increases when CP–CP interactions are strengthened, but decreases when they are weak (Bendahmane *et al*. 1997).

6. WHY DOES CP NOT RESTRICT THE SYSTEMIC SPREAD OF INFECTION?

If the hypothesis that CP interferes with an early event in infection is correct, why does systemic infection occur in plants in which high levels of inoculum are used? There are at least two possible explanations. First, virions that spread the infection systemically may be structurally different from those that initiate infection after mechanical inoculation. Such differences may enable the virus to pass through the early events of infection in systemically infected leaves, by-passing the step at which CP is effective. Second, the level of inoculum that enters the upper, systemically infected leaves is likely to be much higher than that required to cause infection in inoculated leaves and, essentially, hyperinoculates the leaves, thereby overcoming CP-MR.

An interesting result of the studies with mutant CPs was the observation that plants containing CP-Thr42Trp were highly resistant to systemic infection. We conducted a study by grafting CP(+) and CP(−) plants (using each as rootstocks or scions), and then challenged the rootstocks by inoculation with TMV. In these studies, we observed that CP-Thr42Trp in the rootstock conferred high levels of resistance to systemic infection, while wild-type CP and other mutants CPs did not (Bendahmane *et al*. 1997). These results suggest that CP-Thr42Trp interferes with a step in TMV replication, and/or local or systemic spread, as well as blocking the disassembly of TMV. There are several possible explanations for these results. The first is that CP is involved in an unknown manner in local spread of infection and that this is interfered with by mutant CP. This is unlikely, based on the results of Dawson and colleagues that CP is not required for local spread of the TMV infection (Hilf & Dawson 1993). The second possibility is that systemic infection, which requires CP, and presumably virions, is restricted because CP-Thr42Trp, restricts the assembly of virions. A third possibility is that CP-Thr42Trp restricts the replication of TMV by, an as yet, unknown mechanism. One might expect that restricted replication would limit expression of movement protein and CP, thereby restricting local and systemic spread, especially spread to vascular cells for systemic infection. While there is to date no clear evidence to support these hypotheses, studies are underway to test a range of possible explanations for the increased resistance that is conferred by CP-Thr42Trp.

7. WHERE DO WE GO FROM HERE?

Based upon the results of studies with mutant CP summarized above, we are confident that such studies will lead to greater understanding of CP-MR as well as to greater levels of resistance in transgenic plants. It is gratifying to make the connection between molecular structure and function of the CP and to begin to apply protein design to a gene for disease resistance. These principles lead one to ask whether or not it will be possible to design a single CP that can confer resistance to widely different tobamoviruses. Such studies will be greatly aided by recent studies which have determined the particle structure of the tobamoviruses tobacco mild green mottle virus (Pattanayek & Stubbs 1992), ribgrass mosaic virus (Wang *et al*. 1997), and cucumber green mottle mosaic virus (Wang & Stubbs 1994). While the overall architectures of these tobamoviruses and TMV are similar, they differ in certain aspects of the shape of the CP and surface features of the virions. It is presumed that such differences are responsible for the lack of resistance to distantly related tobamoviruses of transgenic plants that contain the TMV-U1 CP. It will be important to determine whether or not TMV CP can be appropriately modified to confer resistance to a wide range of tobamoviruses, as well as to TMV. Similarly, as we have shown that CP mutant Thr42Trp has an unexpected effect on systemic infection of transgenic plants, it is likely that these and other studies will reveal as yet unknown functions of the CP in regulating the imitation of virus infection, replication, and

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local and systemic virus spread within the plant. This information will be very useful in agricultural biotechnology as well as in studies in basic virology.

The work on coat-protein-mediated resistance would not have been possible without the prior history of scientific discoveries made by plant pathologists and virologists through the past century. Many, but not all, of these discoveries are presented in other articles in this volume. Special recognition is given to Professor Milton Zaitlin, with whom I studied as a post-doctoral fellow. I owe an enormous debt of gratitude to my students, post-doctoral associates, and colleagues and collaborators at Monsanto Company, The Scripps Research Institute, and around the world who have participated in this research. Many are co-authors of papers cited. I also acknowledge the support for research on CP-MR provided by the National Institutes of Health (AI27161) and Monsanto Company (1982–1991).

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