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PROGRESS REPORT

In the year since the last Progress Report on the DOE-sponsored project entitled "Mechanisms of Inhibition of Viral Replication in Plants", progress has not been substantial. We really had been working on too many fronts and have spent most of the last year consolidating our gains and reinforcing our results. This was enforced in part by a complete turnover in the personnel of this lab, a reduction in the personnel numbers and the PI going on sabbatical leave for 6 months. During this time period, we have made some progress and also encourntered a few problems.

1. Mapping resistance breakage in the ToMV genome. The work mentioned in the last Progress Report on mapping sequences involved in resistance breakage in tomato by tomato mosaic virus (ToMV) has been published (Calder & Palukaitis, 1992).

2. Transgenic plants and viral movement genes.

a. ToMV movement gene. There has been no work done on this project since the end of July 1991, when the postdoc working on this project, Dr. Victoria Calder, returned to New Zealand. The new postdoc, Dr. Igor Kaplan, has harvested fruit from the R_0 generation transgenic tomato plants and collected the R_1 seeds. Most of the fruit have few if any seeds, so this has been an ongoing project, although not a fruitless one. We now have seeds from the tomato lines Vendor and Vendor containing the *Tm-2* resistance gene; both lines with either the Rok 2 transformation vector alone, or containing the tobacco mosaic virus (TMV) movement gene. (This was confirmed using protein blot analysis by Dr. Lee Zhang, a postdoc working on another grant, using antisera provided by Dr. Roger Hull, Norwich, UK.) Unfortunately, the germination frequency of these transgenic seeds is not very high, and so we haven't done any additional experiments with them. We had planned to continue this work this fall with the aid of a new student, but for technical reasons, he cannot come, and so we will soon be reallocating responsibility for this project.

b. CMV movement gene. We have collected R_2 generation seeds from the R_1 generation transgenic plants expressing the cucumber mosaic virus (CMV) movement (3a) gene that were described in last years Progress Report, and we have made crosses

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with transgenic plants expressing the CMV coat protein gene. These various plants will be used in future studies. We have also constructed a series of deletion mutants in RNA 3 of CMV, in both the 3a (movement) gene and the coat protein gene, to test on the above transgenic plants, for complementation of function. These tests will be done in the near future. We were hoping to first demonstrate unequivocally that these plants produced the CMV movement protein. This, however, has not been possible. We obtained a monoclonal antiserum to the CMV 3a protein, but this antiserum does not detect the 3a protein on western blots (even by those supplying the serum). Dr. Kaplan attempted to prepare a polyclonal antiserum against a fusion protein. This antiserum reacted against bacterial heat shock proteins and some other plant proteins, but not against the CMV 3a protein. Currently, we are having several oligopeptides custom-synthesized (and coupled to another protein) for production of a CMV 3a specific antiserum. We hope to complete this phase of the project in the next few months. Then, further work on the resistance observed to tobamovirus movement and complementation of CMV movement can continue.

3. Replication of tobamoviruses in tomato protoplasts. This work has been discontinued temporarily, pending completion of the work in section 2.a of this report, and the arrival of new personnel.

4. Transgenic plants and CMV coat protein. This work was repeated by a new graduate student, Mr. Qiubo Li, who obtained similar (and equally bizarre) results to those reported in the previous Progress Report. All that work was with the R_1 generation of transgenic tobacco from 5 lines expressing the Fny-CMV coat protein gene. Subsequent work by Mr. Li, on the resistance of such plants to CMV infection, using R_2 generation plants was not consistent from experiment to experiment, indicating the segregation of additional, unknown factors. Thus, for the present, we have discontinued this line of research.

Tomato plants transgenic for the coat protein gene of the M-strain of CMV have been produced and the seed collected. However, no tests have as of yet been undertaken.

5. Mapping CMV sequences involved in virus replication/resistance breakage.

a. CMV RNA 3. The work described on mapping the sequences in M-CMV RNA 3 that are involved in eliciting a resistance response in *Cucurbita pepo* (zucchini squash)

has been on hiatus since the last Progress Report, when Dr. Shintaku left this laboratory. There has not been any more progress on the structural determination of the CMV particle using X-ray diffraction of crystal. Finally, Dr. Hanada (in Japan) has changed jobs, also leading to a delay in his part of the project concerned with making full-length cDNA clones of the LS-strain of CMV. Recently, however, Dr. Zhang has made such constructs in this laboratory and currently we are assessing their biological activitiy.

b. CMV RNA 2. The work involving mapping resistance breakage in legumes and maize has been discontinued as part of this project. With the construction of biologically active cDNA clones of the LS-CMV RNAs, resistance breakage in *Lactuca saligna* (wild lettuce) will instead be mapped.

c. CMV RNA 1. We have likewise constructed a full-length cDNA of LS-CMV RNA 1 and Dr. Zhang has made some recombinants with Fny-CMV RNA 1. These have not yet been assessed for biological viability.

A manuscript mapping temperature-sensitivity to CMV replication in *Cucumis melo* (muskmelon) was published (Roossinck, 1992).

6. Mapping pathogenicity domains.

a. CMV RNA 3. The work delimiting the chlorosis-inducing domain by a comparison of coat protein genes of CMV strains that induce systemic chlorosis and those strains that do not, has been published (Shintaku, 1991). Similarly, the work describing the localization of this phenotypic domain to amino acid 129 of the CMV coat protein is in press in the Plant Cell (Shintaku *et al.*, 1992).

b. Satellite RNAs. The mapping of the host-specific, chlorosis-regulating domain was published in the Plant Journal (Sleat and Palukaitis, 1992).

Several reviews on the structure and function analysis of satellite RNAs of plant viruses (Roossinck *et al.*, 1992) mapping functional domains in CMV and its satellite RNAs (Palukaitis *et al.*, 1991), and the (molecular) biology of CMV (Palukaitis *et al.*, 1992) have all been published.

7. Identifying the aphid transmission domain. The amino acid sequences in the M-CMV coat protein gene that render the virus aphid non-transmissible are still under analysis. We have demonstrated that amino acid 129 (the chlorosis domain) is in part involved, but amino acid 168 is probably the major determinant. Mutagenesis of position 168 from the

cysteine present in M-CMV to the tyrosine observed at that position has been done (by Dr. Zhang) and the aphid transmission tests are being done (by our collaborator, Dr. Keith Perry). We have already demonstrated that mutagenesis from cysteine to either phenylalanine (present in the non-transmissible strain C-CMV) or to threonine does not lead to transmissibility. If this last experiment restores transmissibility, then this project will be completed.

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