77

BARD

FINAL REPORT

PROJECT No. I-6-79

**Plant Protection by a Plasmid Induced
Killer System from a Plant Pathogen**

Y. Koltin, R.F. Bozarth

1983

ISRAEL

B/ No. of Proposal- 1-6-79

Title of Proposed Research: Plant Protection by a Plasmid Induced Killer System from a Plant Pathogen

Name(s) of Investigator(s)

Principal Investigator: Prof. Yigal Koltin

Cooperating Investigator: Prof. R.F. Bozarth

Name and Address of Affiliated Institution(s):

Grantee Institution: Department of Microbiology, Faculty of Life Sciences
Tel Aviv University

Cooperating Institution: Department of Biological Sciences, State University
of Indiana at Terre Haute, Terre Haute, Indiana

Project Starting Date: November 1, 1979

Type of Report: Initial ___ Comprehensive ___ Final x

Signature

Principal Investigator

Y. Koltin

Institution Authorizing Official

POOR ORIGINAL

ABSTRACT

The study was intended to explore and develop an unconventional approach to plant protection through the application of a viral genome that encodes a toxin that is effective against a number of plant pathogens. The purpose was to use the viral genome by molecular cloning as new information that should be introduced to plant cells and confer these cells with a new type of resistance.

Among the wild population of the corn smut Ustilago maydis some strains secrete a proteinaceous toxin that kills cells of sensitive strains of the same species and of other Ustilaginales. The phenomenon is known as the "killer phenomenon" and is due to the presence of specific viruses in killer strains. The characterization of this phenomenon and both the toxin and the virus associated with this phenomenon stimulated the idea that through genetic engineering the viral information may be used for plant protection. The idea stemmed from the fact that the toxin specificity extended beyond the host range of each pathogen. As an example, the virus encoded toxin expressed by strains of Ustilago maydis the pathogen of corn is also effective against U. hordei the pathogen of barley, U. avenae the pathogen of oats and many other Ustilaginales. The distinct species of the pathogens share no hosts. Therefore, if the viral information encoding the toxin could be transferred in a stable form into cells of barley or oats their pathogens will be confronted with a host producing a toxin effective against these pathogens. Such transfer could be achieved in a stable form via genetic cloning of the viral information once this information was fully characterized. This approach should offer a model for future studies perhaps in a less complex situation for genetic manipulation of competing microorganisms.

The research dealt primarily with (a) the characterization of the viral genome by deletion mapping and in vitro translation of its segmented genome (b) characterization of the physical organization of the segmented genome and its mode of expression and (c) the cloning of the viral information.

The results defined the distribution of the viral functions of the different segments and the functional relations and dependence among the segments. The genetic mapping based on studies of deletion mutants and derived hybrids was substantiated by in vitro translation. The physical organization of the complex genomes of these double stranded RNA viruses indicated that each segment class is separately encapsidated and the entire genome operates as a helper virus system in which only one segment class can be maintained while the rest are dependent for maintenance on this class. Furthermore, each capsid is associated with a transcriptase but the source of this enzyme may be either from the heavy segments of the viral genome or as a nuclear function. However, the information obtained indicated specifically the segments that will have to be cloned to obtain the expression of the toxin. The characterization of the virus associated transcriptase opened a direct approach to the cloning of the viral information with the dispensation of the need to clone dsRNA which is most difficult in cases of segments with a high melting point. The study has reached a point that should allow the cloning of the viral genes in lower microorganisms within a very short time.

Genetic mapping of the viral genome

All 3 complexes of the dsRNA viruses of Ustilago maydis associated with the secretion of a proteinaceous toxin consist of a segmented genome. The segments range from 4.2×10^6 daltons to 0.3×10^6 daltons. The segments can be classified into 3 major classes based on size (see description in attached articles). Since the goal of the study was aimed at cloning the sequence associated with the expression of the toxin a functional assignment to each segment was essential to determine what is the essential information for toxin expression. The enclosed article "Assignment of functions to segments of the dsRNA genome of the Ustilago virus" and "Mapping the immunity function of the Ustilago maydis P1 virus" constitute a genetic approach to the mapping of viral functions. These studies were based on the isolation of mutants with incomplete genomes lacking specific segments and the generation by crosses of hybrid genomes. These studies suggested that the heavy segments (4.1 to 1.8×10^6 daltons) are associated with virus replication and coat formation, the medium size segments (1.0 to 0.5×10^6 daltons) are associated with toxin expression and in some viruses may be involved also in expression of immunity to the toxin. The smallest molecules in the complex provides immunity against the toxin to at least one of the 3 toxins produced by the different virus complexes of Ustilago.

Thesis

Sylvia Simon. 1981. Mapping the Viral Genome of Ustilago maydis by in vitro Translation. M.Sc. Thesis, Department of Microbiology, Tel Aviv University.

Articles and Manuscripts

- ✓ 1. Koltin, Y., R. Levine and A. Tamarkin. 1980. Assignment of functions to segments of the dsRNA genome of the Ustilago virus. Molec. Gen. Genetics 178, 173-178.
- ✓ 2. Koltin, Y. and R. Steinlauf. 1980. The killer phenomenon in Ustilago: Electron microscopy of the dsRNA encapsidated in individual virus particles. Arch. Microbial. 128, 45-52.
- ✓ 3. Bozarth, R.F., Y. Koltin, M.B. Weisman, R.L. Parker, R.E. Dalton and R. Steinlauf. 1981. The molecular weight and packaging of dsRNAs in the mycovirus from Ustilago maydis killer strains. Virology 113, 492-502.
- ✓ 4. Simon, S., M. Gorecki and Y. Koltin. 1981. In vitro translation of the Ustilago virus dsRNA. Genetics 97: s76.
- ✓ 5. Peery, T., Y. Koltin and A. Tamarkin. 1982. Mapping the immunity function of the Ustilago maydis P1 virus. Plasmid 7, 52-58.
- ✓ 6. Ben-Zvi, B.S. and Y. Koltin. RNA polymerase activity in virions from Ustilago maydis. Molec. Cell Biol. (in press).

TABLE OF CONTENTS

	Page
Abstract	1
Genetic mapping of viral genome	3
Functional mapping	4
Genome organization	
Encapsidation of the dsRNA segments	10
Virus associated RNA polymerase	11
Molecular cloning of the viral dsRNA	12
Evaluation of achievements	14
Description of results of cooperation	16
Thesis	17
List of Articles and manuscripts	17