**BARD Final Scientific Report
Cover Page**

Date of Submission of the report: **August 1, 2002**

BARD Project Number: **US-2969-98**

Project Title: Viral and Host Cell Determinants of Nuclear Import and Export of the Tomato Yellow Curl Leaf Virus in Tomato Plants

Investigators

Institutions

Principal Investigator (PI):

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Collaborating Investigators:

none

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Karyopherin alpha, coat protein, homotypic interaction, single-stranded DNA binding

Abbreviations commonly used in the report, in alphabetical order:

CP, coat protein, NES, nuclear export signal, NLS, nuclear localization signal, TYLCV – tomato yellow leaf curl virus

Budget: IS: \$146,000

US: \$119,000

Total: \$265,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)	2	3	1	6
Submitted, in review, in preparation	0	1	2	3
Invited review papers	0	2	2	4
Book chapters	0	1	0	1
Books	0	1	0	1
Master theses	0	0	1	1
Ph.D. theses	0	0	0	0
Abstracts	0	0	0	0
Not refereed (proceedings, reports, etc.)	0	0	0	0

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Tzvi Tzfira,
Shoko Ueki,
Vipin Hallan

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1	0	2
Longer Visits (Sabbaticals)	1	0	0	1

Description of Cooperation:

Both PIs closely collaborated by frequent communication through electronic mail and frequent visits to each other's laboratories. Constant discussions were held, generating ideas and experiments throughout the project. A significant contribution to current research was exchange of genetic material, including constructs prepared in one laboratory and utilized by the other. These everyday contacts led to several joint publications driven by new discoveries, which were made exclusively due of this increased level of collaboration. Both groups freely shared ideas, technologies and materials and the collaboration between the two groups continues. If funded again, it will most probably bring better understanding of the life cycle of TYLCV.

Patent Summary (numbers)

	Israeli inventor (s) only	US inventor (s) only	Joint IS/US inventors	Total
Submitted	0	1	0	1
Issued (allowed)	0	1	0	1
Licensed	0	0	0	0

Viral and Host Cell Determinants of Nuclear Import and Export of the Tomato Yellow Curl Leaf Virus in Tomato Plants

Abstract

Tomato yellow leaf curl geminivirus (TYLCV) is a major pathogen of cultivated tomato, causing up to 100% crop loss in many parts of the world. In Israel, where TYLCV epidemics have been recorded since the 1960's, this viral disease is well known and has been of economic significance ever since. In recent years, TYLCV outbreaks also occurred in the "New World" - Cuba, The Dominican Republic, and in the USA, in Florida, Georgia and Louisiana. Thus, TYLCV substantially hinders tomato growth throughout the world. Surprisingly, however, little is known about the molecular mechanisms of TYLCV interaction with the host tomato cells. The present proposal, a continuation of the project supported by BARD from 1994, expanded our understanding of the molecular mechanisms by which TYLCV enters the host cell nucleus for replication and transcription and exits it for the subsequent cell-to-cell spread. Our project sought two objectives:

I. To study the roles of the viral capsid protein (CP) and host cell factors in TYLCV nuclear import.

II. To study the roles of CP and host cell factors in TYLCV nuclear export.

Our research toward these goals have produced the following major achievements:

- Developed a one-hybrid assay for protein nuclear export and import (#3 in the List of Publications).
- Identified a functional nuclear export signal (NES) in the capsid protein (CP) of TYLCV (#3 in the List of Publications).
- Discovered homotypic interactions between intact TYLCV CP molecules and analyzed these interactions using deletion mutagenesis of TYLCV CP (#5 in the List of Publications).
- Showed developmental and tissue-specific expression of the host factor required for nuclear import of TYLCV CP, tomato karyopherin alpha 1, in transgenic tomato plants (#14 in the List of Publications).
- By analogy to nuclear import of TYLCV, identified an Arabidopsis VIP1 protein that participates in nuclear import of Agrobacterium T-complexes via the karyopherin alpha pathway (#4, 6, and 8 in the List of Publications).

These research findings provided significant insights into (i) the molecular pathway of TYLCV entry into the host cell nucleus, and (ii) the mechanism by which TYLCV is exported from the nucleus for the cell-to-cell spread of infection. Furthermore, the obtained knowledge will help to develop specific strategies to attenuate TYLCV infection, for example, by blocking viral entry into and/or exit out of the host cell nucleus. Also, as much of our findings is relevant to all geminiviruses, new anti-TYLCV approaches developed based on the results of our research will be useful to combat other members of the Geminivirus family. Finally, in addition to the study of TYLCV nuclear import and export, our research contributed to our understanding of general mechanisms for nucleocytoplasmic shuttling of proteins and nucleic acids in plant cells.

Achievements

The main significant achievements of the present project are:

1. Development of a one-hybrid assay for protein nuclear export and import.
2. Identification of a functional nuclear export signal (NES) within TYLCV CP.
3. Discovery and characterization of homotypic interactions between TYLCV CP molecules.
4. Discovery of developmental and tissue-specific expression of the host factor required for nuclear import of TYLCV CP, tomato karyopherin alpha 1, in transgenic tomato plants.
5. Discovery, by analogy to nuclear import of TYLCV, of an Arabidopsis VIP1 protein that participates in nuclear import of Agrobacterium T-complexes via the karyopherin alpha pathway.

1. Development of a one-hybrid assay for protein nuclear export and import

For a simple genetic assay to detect active nuclear import (NLS) and export signals (NES) based on their function within yeast cells, the bacterial LexA protein was modified (mLexA) to abolish its intrinsic NLS and fused to the activation domain of the yeast Gal4p (Gal4AD) with or without the SV40 large T-antigen NLS. In the import assay, if a tested protein fused to mLexA-Gal4AD contains a functional NLS, it will enter the cell nucleus and activate the reporter gene expression. In the export assay, if a tested protein fused to mLexA-SV40 NLS-Gal4AD contains a functional NES, it will exit into the cytoplasm, decreasing the reporter gene expression. We tested this system with known NLS and NES. This approach can help to identify, analyze, and select for other proteins containing functional NLS and NES signals.

2. Identification of a functional nuclear export signal (NES) within TYLCV CP

The pNEA-based nuclear export assay and histidine selection to demonstrate that, in addition to its NLS, TYLCV CP contains a NES functional in yeast. Similarly to another nuclear-exported protein, Rev, a CP fusion substantially decreased histidine prototrophy, indicating reduction in *HIS3* gene expression and, by implication, the presence of an active NES within CP. Next, the CP NES was mapped relative to its known NLS sequences. The CP amino terminus promoted efficient expression of the reporter genes, suggesting that the CP NES is located within the deleted part of the protein, i.e., residues 38 to 260. Deletion of amino acid residues from 38 to 113, on the other hand, did not enhance *HIS3* gene expression. This result indicates that the CP NES is not present in the middle portion of CP. In contrast,

removal of the CP C-terminus (residues 114 to 260) restored *HIS3* expression, suggesting that the deleted C-terminal region contains a functional NES signal. Thus, CP likely contains two types of spatially distant targeting signals, amino terminal and middle NLSs and a C-terminal NES. Because CP NES functions in the pNEA assay within the context of the full-length CP, i.e., in the presence of the amino terminal NLS, this NES signal may dominate the CP NLS signal.

3. Discovery and characterization of homotypic interactions between TYLCV CP molecules

Tomato yellow leaf curl virus (TYLCV) capsid protein (CP) forms the capsule that encapsidates the viral genomic ssDNA. We have analyzed the homotypic interaction capacity of full-length and mutated CP. We found that full-length CP interacts with itself. Truncation of the protein from the C-terminal led to diminution of the self-interaction process. Also, the N-terminal region of the CP seemed to be necessary for the interaction. As the two deletion mutants interacted successfully with the wild-type protein, while they failed to self-interact, we suggest that the N-terminal amino acids interact with amino acids of the C-terminal region. Changes in CP homotypic interaction capacity were detected when mutations in the middle portion of the protein were introduced.

4. Discovery of developmental and tissue-specific expression of the host factor required for nuclear import of TYLCV CP, tomato karyopherin alpha 1, in transgenic tomato plants

The promoter region (2.2 kb upstream from transcription start) of karyopherin α 1 was isolated by us earlier. Here we characterized its mode of expression in transgenic tobacco and tomato plants. In an attempt to study its mode of expression in plants, the promoter was fused to the gene coding for the β -glucuronidase (GUS) and transported into tomato and tobacco cells via *Agrobacterium tumefaciens*. GUS expression was studied in different stages of plant development by screening its expression in different parts of the plant. Surprisingly, the gene was expressed in tissues that accumulation of the plant hormone auxin, is known to occur. For example, expression in a flower is indeed visible in tissues where it is well known that auxin accumulates. This was also shown in tomato fruits where the expression was in the seeds. Again, this tissue contains much auxin in young fruits, suggesting that karyopherin α 1 is regulated by auxin.

Discovery, by analogy to nuclear import of TYLCV, of an Arabidopsis VIP1 protein that participates in nuclear entry of Agrobacterium T-complexes via the karyopherin alpha pathway

T-DNA nuclear import is a central event in genetic transformation of plant cells by Agrobacterium. Presumably, this event is mediated by two bacterial proteins, VirD2 and VirE2, which are associated with the transported T-DNA molecule. While VirD2 is imported into the nuclei of plant, animal, and yeast cells, nuclear uptake of VirE2 occurs most efficiently in plant cells. To better understand the mechanism of VirE2 action, a cellular interactor of VirE2 was identified and its encoding gene cloned from Arabidopsis. The identified plant protein, designated VIP1, specifically bound VirE2 and allowed its nuclear import in non-plant systems. In plants, VIP1 was required for VirE2 nuclear import and Agrobacterium tumorigenicity, participating in early stages of T-DNA expression. Furthermore, our results indicated that VIP1 is imported into the plant cell nucleus via the karyopherin alpha-dependent pathway, and that elevated intracellular levels of VIP1 render the host plants significantly more susceptible to transient and stable genetic transformation by Agrobacterium, probably due to the increased nuclear import of the T-DNA.

Cooperation

A significant cooperation was implemented throughout the entire project period. The US and the Israeli PIs were in constant contact by e-mail and phone and several visits to each other labs were arranged. The US PI also spend a sabbatical in Israel and had an opportunity to visit the Israeli PI lab on a daily basis. besides these contacts and discussions, the collaborating labs exchanged genetic material, i.e., expression constructs, which greatly facilitated experiments by both groups. Close cooperation between the PIs and the significant findings of this project were instrumental in generating another successful BARD-funded research by the same groups as well as establishment of an applied research program headed by Dr. Gafni aimed at preventing geminiviral infection in planta and funded by the Chief Scientist of the Ministry of Agriculture of Israel and by the Ministry of Science and technology of Israel.

List of Publications

1. Tzfira, T. & Citovsky, V. (2000) From host recognition to T-DNA integration: the function of bacterial and plant genes in the Agrobacterium-plant cell interaction. *Mol. Plant Pathol.* **1**, 201-212.
2. Tzfira, T., Rhee, Y., Chen, M.-H., Kunik, T., & Citovsky, V. (2000) Nucleic acid transport in plant-microbe interactions: the molecules that walk through the walls. *Annu. Rev. Microbiol.* **54**, 187-219.
3. Rhee, Y., Gurel, F., Gafni, Y., Dingwall, C., & Citovsky, V. (2000) A genetic system for detection of protein nuclear import and export. *Nature Biotechnol.* **18**, 433-437.
4. Tzfira, T. & Citovsky, V. (2001) Comparison between nuclear import of nopaline- and octopine-specific VirE2 protein of Agrobacterium in plant and animal cells. *Mol. Plant Pathol.* **2**, 171-176.
5. Hallan, V. & Y. Gafni (2001) Tomato yellow leaf curl virus (TYLCV) capsid protein (CP) subunit interactions: implications for viral assembly. *Arch Virol.* **146**, 1765-1773.
6. Tzfira, T., M. Vaidya, & V. Citovsky (2001) VIP1, an Arabidopsis protein that interacts with Agrobacterium VirE2, is involved in VirE2 nuclear import and Agrobacterium infectivity. *EMBO J.* **20**, 3596-607.
7. Kunik, T., Tzfira, T., Kapulnik, Y., Gafni, Y., Dingwall, C., & Citovsky, V. (2001) Genetic transformation of HeLa cells by Agrobacterium. *Proc. Natl. Acad. Sci. USA* **98**, 1871-1876.
8. Tzfira, T., Vaidya, M., & Citovsky, V. (2002) Increasing plant susceptibility to Agrobacterium infection by overexpression of the Arabidopsis *VIP1* gene. *Proc. Natl. Acad. Sci. USA* **99**, in press.
9. Gafni, Y. & Epel, B. (2002) The role of host and viral proteins in intra- and inter-cellular trafficking of geminiviruses. *Physiol. Mol. Plant Pathol.* **60**, 231-241.
10. Gafni, Y. (2002) *Tomato yellow leaf curl virus*, the intra cellular dynamics of a plant DNA virus. *Mol. Plant Pathol.* **3**, in press.
11. *Nuclear Import and Export* (2003) (T. Tzfira and V. Citovsky, eds.) Landes Bioscience, Georgetown, TX, in press.
12. Tzfira, T. & Citovsky, V. (2003) Nuclear import of Agrobacterium T-DNA. In *Nuclear Import and Export* (T. Tzfira and V. Citovsky, eds.) Landes Bioscience, Georgetown, TX, in press.
13. Gafni, Y., Dabush, D., Cohen, Y. & Mizrachy, L. (2002) Cloning and functional

characterization of the tomato karyopherin α gene promoter. In preparation.

14. Tzfira, T., Mayda, E., & Citovsky, V. (2002) Nuclear import of VirF, a host range factor of *Agrobacterium*, in plant cells. In preparation..
15. Spanov, H. & Gafni, Y. (2002) Characterization of the nuclear export signal of TYLCV CP. In preparation.

The BARD support was instrumental in development of three inventions for which the following patents were applied/issued:

1. Rhee, Y. & Citovsky, V. (1999) “Genetic Assay for Protein Nuclear Import”. U.S. patent # 09/435,274
2. Tzfira, T. & Citovsky, V. (2000) “Production of Plants Resistant and Super-Susceptible to Genetic Transformation by *Agrobacterium*”. Application for U.S. patent filed in August 2000

Appendix

Table of Contents:

1. Reviews published and in press
2. pPapers published and in press