Curriculum Vitae

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Academic Positions

Associate Professor: Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY 11794-5215
Fields: Nuclear transport of proteins and nucleic acids in plants, cell-to-cell and systemic movement of plant virus nucleic acids, intercellular communication and macromolecular trafficking in plants. June 1999 – present

Assistant Professor: Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY 11794-5215 November 1993 – May 1999

Education

Postdoctoral Fellow: Department of Plant Biology, University of California, Berkeley, CA 94720
Fields: Analysis of proteins mediating transfer of T-DNA from *Agrobacterium* to plants, nuclear transport of proteins and protein-nucleic acid complexes in plants, biochemistry and molecular biology of plasmodesmata and cell-to-cell movement of plant virus nucleic acids
June 1987 - August 1993 Advisor: Dr. Patricia Zambryski

Ph.D. studies: Department of Biological Chemistry, Hebrew University, Jerusalem, Israel 91904
Thesis: Early stages in the process of virus entry: interaction of viral envelope glycoproteins with biological and artificial membranes
November 1983 - May 1987 Advisor: Dr. Abraham Loyter

M.Sc. studies: Department of Biological Chemistry, Hebrew University, Jerusalem, Israel 91904
Thesis: Membrane dynamics of antibodies and lectins in keratinocytes: transmembrane signalling in ligand-induced cytotoxicity
September 1981 - August 1982 (*cum laude*) Advisor: Dr. Yoram Milner

B.Sc. studies: Faculty of Natural Sciences, Hebrew University, Jerusalem, Israel 91904
Major: Biology September 1978 - June 1981

Editorial Service

Member of Editorial Board of *Molecular Plant Pathology*, 1999-present

Awards

Lady Davis Research Fellowship, 2000-2001
Profiled in Marquis "Who'sWho in Science and Engineering 2000-2001"
Dr. Chaim Weizmann Postdoctoral Fellowship. 1987-1989
Graduate Fellowship of the Dean from the Hebrew University of Jerusalem, 1983-1986

Invited Speaker

11th Annual Meeting of the American Society for Virology, Ithaca, NY (1992)
2d International Workshop on Plasmodesmatal Biology, Oosterbek, The Netherlands (1992)
Molecular Genetics of Plant-Microbe Interactions Symposium, New Branswick, NJ (1993)
Biotechnology Research for Innovation, Development and Growth in Europe (BRIDGE)
Final Sectoral Meeting on Plant-Microbe Interactions, Dourdan, France (1994)
Association of Applied Biologists Meeting, Cambridge, UK, March 1995
137th Meeting of the Society for Microbiology, Edinburgh, Scotland, UK (1997)
IX International Congress on Plant Tissue and Cell Culture, Jerusalem, Israel (1998)
Tobacco Mosaic Virus: Pioneering Research for a Century, Edinburgh, Scotland, UK (1998)
13th John Innes Symposium on "Attack and Defense in Plant Disease", Norwich, UK (1999)
Plasmodesmata 2000, Durban, South Africa (2000)

List of Publications

Peer-Reviewed Research Articles

1. Citovsky, V., Neistadt, S., Michel, B., & Milner, Y. (1983) Surface dynamics of bound IgG on guinea pig keratinocytes: Possible relation to Pemphigus-IgG-induced cell death. *J. Invest. Dermatol.* **80**, 366.

2. Citovsky, V. & Loyter, A. (1985) Fusion of Sendai virions or reconstituted Sendai virus envelopes with liposomes or erythrocyte membranes lacking virus receptors. *J. Biol. Chem.* **260**, 12072-12077.

3. Citovsky, V., Blumenthal, R., & Loyter, A. (1985) Fusion of Sendai virions with phosphatidylcholine-cholesterol liposomes reflects the viral activity required for fusion with biological membranes. *FEBS Lett.* **193**, 135-140.

4. Citovsky, V., Yanai, P., & Loyter, A. (1986) The use of circular dichroism to study conformational changes induced in Sendai virus envelope glycoproteins: A correlation with the viral fusogenic activity. *J. Biol. Chem.* **261**, 2235-2239.

5. Citovsky, V., Zakai, N., & Loyter, A. (1986) Specific requirement for liposome associated sialoglycolipids, but not sialoglycoproteins, to allow lysis of phospholipid vesicles by Sendai virions. *Exp. Cell Res.* **166**, 279-294.

6. Citovsky, V., Laster, Y., Schuldiner, S., & Loyter, A. (1987) Osmotic swelling allows fusion of Sendai virions with membranes of desialized erythrocytes and chromaffin granules. *Biochemistry* **26**, 3856-3864.

7. Citovsky, V., Rottem, S., Nussbaum, O., Laster, Y., Rott, R., & Loyter, A. (1988) Animal viruses are able to fuse with prokaryotic cells. *J. Biol. Chem.* **263**, 461-467.

8. Citovsky, V., De Vos, G., & Zambryski, P. (1988) Single-stranded DNA binding protein encoded by the virE locus of Agrobacterium tumefaciens. *Science* **240**, 501-504.

9. Citovsky, V., Wong, M. L., & Zambryski, P. (1989) Cooperative interaction of Agrobacterium VirE2 protein with single stranded DNA: Implications for the T-DNA transfer process. *Proc. Natl. Acad. Sci. USA* **86**, 1193-1197.

10. 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**, 637-647.

11. Citovsky, V., Knorr, D., & Zambryski, P. (1991) Gene I, a potential movement locus of CaMV, encodes an RNA binding protein. *Proc. Natl. Acad. Sci. USA* **88**, 2476-2480.

12. Howard, E., Zupan, J., Citovsky, V., & Zambryski, P. (1992) The VirD2 protein of Agrobacterium tumefaciens contains a C-terminal bipartite nuclear localization signal: implications for nuclear uptake of DNA in plant cells. *Cell* **68**, 109-118.

13. Citovsky, V., Wong, M.L, Shaw, A., Prasad, B.V.V., & Zambryski, P. (1992) Visualization and characterization of tobacco mosaic virus movement protein binding to single-stranded nucleic acids. *Plant Cell* **4**, 397-411.

14. Citovsky, V., Zupan, J., Warnick, D., & Zambryski, P. (1992) Nuclear localization of Agrobacterium VirE2 protein in plant cells. *Science* **256**, 1802-1805.

15. Citovsky, V., McLean, B.G., Zupan, J., & Zambryski, P. (1993) Phosphorylation of tobacco mosaic virus cell-to-cell movement protein by a developmentally regulated protein kinase associated with the plant cell wall. *Genes & Dev.* **7**, 904-910.

16. Waigmann, E., Lucas, W., Citovsky, V., & Zambryski, P. (1994) Direct functional assay for tobacco mosaic virus cell-to-cell movement protein and identification of a domain involved in increasing plasmodesmal permeability. *Proc. Natl. Acad. Sci. USA* **91**, 1433-1437.

17. Citovsky V., Warnick, D., & Zambryski, P. (1994) Nuclear import of Agrobacterium VirD2 and VirE2 proteins in maize and tobacco. *Proc. Natl. Acad. Sci. USA* **91**, 3210-3214.

18. Zupan, J., Citovsky, V. & Zambryski, P. (1996) Agrobacterium VirE2 imports DNA into the plant cell nucleus. *Proc. Natl. Acad. Sci. USA* **93**, 2392-2397.

19. Guralnick, B., Thomsen, G. & Citovsky, V. (1996) Transport of DNA into the nuclei of Xenopus oocytes by a modified VirE2 protein of Agrobacterium. *Plant Cell* **8**, 363-373.

20. Gafni, Y., Kunik T., Czosnek, H., & Citovsky, V. (1997). Transgenic tomato plants expressing TYLCV capsid protein are resistant to the virus: the role of the nuclear localization signal (NLS) in the resistance. *Acta Hort.* **447**, 387-391.

21. Citovsky, V., Guralnick, B., Simon, M., & Wall, J., (1997) The molecular structure of Agrobacterium VirE2-single stranded DNA complexes involved in nuclear import. *J. Mol. Biol.* **272**, 718-727.

22. Lartey, R., Ghoshroy, S., Hoe, J., & Citovsky, V. (1997) Movement and subcellular localization of a tobamovirus in Arabidopsis. *Plant J.* **12**, 537-545.

23. Ballas, N. & Citovsky, V. (1997) Nuclear localization signal binding protein from Arabidopsis mediates nuclear import of Agrobacterium VirD2 protein. *Proc. Natl. Acad. Sci. USA* **94**, 10723-10728.

24. Kunik, T., Palanichelvam, K., Czosnek, H., Citovsky, V., & Gafni, Y. (1998) Nuclear import of the capsid protein of tomato yellow leaf curl virus (TYLCV) in plant and insect cells. *Plant J.* **13**, 393-399.

25. Ghoshroy, S., Feldman, K., & Citovsky, V. (1998) Inhibition of plant viral systemic movement by non-toxic concentrations of cadmium. *Plant J.* **13**, 591-602.

26. Lartey, R., Ghoshroy, S., & Citovsky, V. (1998) Identification of an *Arabidopsis thaliana* mutation (*vsm1*) that restricts systemic movement of tobamoviruses. *Molec. Plant-Microbe Interact.* **7**, 706-709.

27. Sheng, J., Lartey, R., Ghoshroy, S., & Citovsky, V. (1998) An Arabidopsis mutant with virus-inducible phenotype. *Virology* **249**, 119-128.

28. Citovsky, V., Ghoshroy, S., Tsui, F., & Klessig, D.F. (1998) Non-toxic concentrations of cadmium inhibit tobamoviral systemic movement by a salicylic acid-independent mechanism. *Plant J.* **16**, 13-20.

29. Ghoshroy, S. & Citovsky, V. (1998) Preservation of plant cell ultrastructure during immunolocalization of virus particles. *J. Virol. Methods* **74**, 223-229.

30. Palanichelvam, K., Czosnek, H., Citovsky, V., and Gafni, Y. (1998) The capsid protein of tomato yellow leaf curl virus binds cooperatively to single-stranded DNA. *J. Gen. Virol.* **79**, 2829-2833.

31. Kunik, T., Mizrachy, L., Citovsky, V., and Gafni, Y. (1999) Characterization of a tomato karyopherin _ that interacts with the tomato yellow leaf curl virus (TYLCV) capsid protein. *J. Exp. Bot.* **50**, 731-732.

32. Chen, M.-H., Ghoshroy, S., Waigmann, E., & Citovsky, V. (1999) Phosphorylation of tobacco mosaic virus cell-to-cell movement protein (MP) regulates viral movement in a host-specific fashion. Submitted

33. Rhee, Y., Gurel, F., Gafni, Y., Dingwall, C., & Citovsky, V. (1999) A genetic system for detection of protein nuclear import and export. Submitted.

Peer-Reviewed Review Articles

1. Loyter, A., Citovsky, V., & Blumenthal, R. (1988) The use of fluorescence dequenching measurements to follow viral membrane fusion events. *Methods Biochem. Analysis* **33**, 129-164.
2. Loyter, A., Chejanovsky, N., & Citovsky, V. (1989) Implantation of isolated carriers and receptors into living cells by Sendai virus envelope-mediated fusion. *Methods Enzymol*. **171**, 829-850.
3. Citovsky, V., Shoshani-Gilad, R., & Loyter, A. (1990) Involvement of osmotic forces in fusion of enveloped viruses with human erythrocytes: studies with Sendai and influenza virus particles. *Prog. Clin. Biol. Res*. **343**, 133-145.
4. Howard, E. & Citovsky, V. (1990) The emerging structure of the Agrobacterium T-DNA transfer complex. *BioEssays* **12**, 103-108.
5. Citovsky, V. & Zambryski, P. (1991) How do plant virus nucleic acids move through intercellular connections? *BioEssays* **18**, 373-379.
6. Citovsky, V. & Zambryski, P. (1993) Transport of nucleic acids through membrane channels: snaking through small holes. *Annu. Rev. Microbiol*. **47**, 167-197.
7. McLean, B.G., Waigmann, E., Citovsky, V., & Zambryski, P. (1993) Cell-to-cell movement of plant viruses. *Trends Microbiol.* **1**, 105-109.
8. Citovsky, V. (1993) Probing plasmodesmal transport with plant viruses. *Plant Physiol.* **102**, 1071-1076.
9. Citovsky, V. & Zambryski, P. (1995) Transport of protein-nucleic acid complexes within and between plant cells. *Membr. Prot. Transport* **1**, 39-57.
10. Sheng, J. & Citovsky, V. (1996) Agrobacterium-plant cell interaction: Have virulence proteins - will travel. *Plant Cell* **8**, 1699-1710.
11. Ghoshroy, S., Lartey, R., Sheng, J., & Citovsky, V. (1997) Transport of proteins and nucleic acids through plasmodesmata. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **48**, 27-49.
12. Lartey, R. & Citovsky, V. (1997) Nucleic acid transport in plant-pathogen interactions. *Genet. Eng*. **18**, 201-214.
13. Creager, A., Scholthof, K.-B., Citovsky, V., & Scholthof, H. (1999) Tobacco mosaic virus: Pioneering research for a century. *Plant Cell* **11**, 301-308.
14. Citovsky, V. (1999) Tobacco mosaic virus: A pioneer of cell-to-cell movement. *Phil. Trans. Royal Soc. Lond.* **354**, 637-643.
15. Tzfira, T., Rhee, Y., Chen, M.-H., & Citovsky, V. (2000) Nucleic acid transport in plant-microbe interactions: The molecules that walk through the walls. *Annu. Rev. Microbiol*. **54**, in press.
16. Rhee, Y., Tzfira, T., Chen, M.-H., Waigmann, E., & Citovsky, V. (2000) Cell-to-cell movement of tobacco mosaic virus: Enigmas and explanations. Molec. Plant Pathol. 1, in press.

Book Chapters

1. Loyter, A., Nussbaum, O., & Citovsky, V. (1988) Active function of membrane receptors in fusion of enveloped viruses with cell plasma membranes. In *Molecular Mechanisms of Membrane Fusion* (S. Ohki, D. Doyle, T.D. Flanagan, S.W. Hui and E. Mayhew, eds.) Plenum Press, p. 413-426.
2. Citovsky, V., Howard, E., Winsor, B., & Zambryski, P. (1989) Proteins that mediate DNA transfer by Agrobacterium tumefaciens to plant cells: summary and perspectives. *UCLA Symp. Mol. Cell. Biol. News Ser*. **101**, 3-18.
3. Howard, E., Citovsky, V., & Zambryski, P. (1990) The T-complex of Agrobacterium tumefaciens. *UCLA Symp. Mol. Cell. Biol. New Ser.* **129**, 1-11.
4. Loyter, A. & Citovsky, V. (1991) The role of envelope glycoproteins in the fusion of Sendai virus with liposomes. In *Membrane Fusion* (J. Wilschut and D. Hoekstra, eds.) Marcel Dekker, Inc. New York., p. 375-393.
5. Loyter, A., Citovsky, V., & Ballas, N. (1991) Sendai virus envelopes as a biological carrier: Reconstitution, targeting and application. In *Membrane Fusion* (J. Wilschut and D. Hoekstra, eds.) Marcel Dekker, Inc. New York., p. 731-749.
6. Citovsky, V., McLean, B.G., Greene, E., Howard, E., Kuldau, G., Thorstenson, Y., Zupan, J., & Zambryski, P. (1991) Agrobacterium-plant cell.interaction: Induction of vir genes and T-DNA transfer. In *Molecular Signals in Plant-Microbe Communications* (D.P.S. Verma, ed.) CRC Press, Inc., p. 169-199.
7. McLean, B.G., Thorstenson, Y., Citovsky, V., Zupan, J., Greene, E., & Zambryski, P. (1993) A. tumefaciens T-DNA transport: Roles for VirB, VirD2 and VirE2. In *Advances in Molecular Genetics of Plant-Microbe Interactions* (E.W. Nester and D.P.S. Verma, eds.) Kulwer Acad. Publ., vol. **2**, p. 63-71.
8. Citovsky, V. (1994) Visualizing protein import into the plant cell nucleus. In *Plant Molecular Biology Manual* (S. Gelvin, R. Schilperoort, D.P.S. Verma, eds.) Kluwer Acad. Publ.,pp. 1-16.
9. Lartey, R., Ghoshroy, S., Sheng, J., & Citovsky, V. (1997) Transport through plasmodesmata and nuclear pores: Cell-to-cell movement of plant viruses and nuclear import of Agrobacterium T-DNA. In *Molecular Aspects of Host-Pathogen Interaction* (M.A. McCrae, J.R. Saunders, C.J. Smith, and N.D. Stow, eds.) Cambridge University Press, pp. 253-280.
10. Kunik, T., Palanichelvam, Mizrachy, L., Czosneck, H., Citovsky, V., and Gafni, Y. (1999) Nuclear import of the capsid protein of tomato yellow leaf curl virus (TYLCV) in plant cells. In *Plant Biotechnology and In Vitro Biology in the 21st Century* (A. Altman, M. Ziv, S. Izhar, eds.) Kluwer Acad. Publ., pp.411-415.
11. Citovsky, V. (1999) Cell-to-cell movement of tobacco mosaic virus. In *Plant Biotechnology and In Vitro Biology in the 21st Century* (A. Altman, M. Ziv, S. Izhar, eds.) Kluwer Acad. Publ., pp.359-363.

Gene Register Reports

1. Ballas, N. & Citovsky, V. (1997) AtKAP_ gene from Arabidopsis (Accession No. U69533) encodes a protein that mediates nuclear import of Agrobacterium VirD2 protein (PGR97-129). *Plant Physiol*. **115**, 314.

Book Reviews

1. Citovsky V. (1994) Arabidopsis: An Atlas of Morphogenesis and Development (J. Bowman, ed.). *Quart. Rev. Biol.* **69**, 526.

2. Citovsky V. (1996) Methods in Plant Molecular Biology (P. Maliga, D. Klessig, A.R. Cashmore, W. Gruissem, J.E. Varner, eds.). *Quarterly Review of Biology* **71**, 280.

3. Citovsky V. (1998) *Arabidopsis* Protocols. (J. M. Martinez-Zapater and J. Salinas, eds.). *Quart. Rev. Biol.* **73**, 345.

4. Citovsky V. (1998) Differentially Expressed Genes in Plants: A Bench Manual. (E. Hansen and G. Harper, eds.). *Quart. Rev. Biol.* **73**, 346.

5. Citovsky V. (1998) Dictionary of Plant Genetics and Molecular Biology. (G. S. Miglani, ed.). *Quart. Rev. Biol.* **74**, 81-82.

6. Citovsky V. (1999) Plant Virology Protocols. (G. D. Foster and S. C. Taylor, eds.). *Quart. Rev. Biol.* **75**, in press.

Patents

1. Citovsky V. (1998) "Protein-Mediated Nuclear Import of DNA". U.S. patent # 08/824,151

2. Citovsky V. (1998) "Genetic Assay for Protein Nuclear Import". Application for a U.S. patent filed in November, 1998.

Current Research Projects

Since I joined the Department of Biochemistry and Cell Biology at Stony Brook in late 1993, I have established a small but vigorous and well-funded laboratory comprising seven postdocs, two technicians and one graduate student. This group of young people is engaged in what I feel are some of the most exciting projects in plant biology. Specifically, we are investigating macromolecular transport between and within cells during host-pathogen interactions. Intercellular traffic is studied on the example of cell-to-cell and systemic movement of plant viruses whereas intracellular transport is examined using nuclear import of *Agrobacterium* T-DNA and nucleo-cytoplasmic shuttling of geminiviruses during the infection process.

Because pathogenic microorganisms often adapt existing cellular machinery for their own needs, plant viruses and *Agrobacterium* likely employ host cell pathways for intercellular and nuclear transport, representing convenient model systems to study these events. Thus, our research not only helps to better understand plant-pathogen interactions but also sheds new light on molecular mechanisms of such general biological processes as transport of nucleic acids and proteins across cell boundaries and through nuclear pores. Below, I summarize our major findings in these areas of research.

I. Viral cell-to-cell and systemic movement and development of viral disease

Communication and molecular transport between plant cells often occur through specialized intercellular connections, the plasmodesmata. Plasmodesmata are also utilized by plant viruses for cell-to-cell movement. Following infection, plant viruses, the "pirates of plasmodesmata", spread from cell to cell until they reach the plant vasculature. Having crossed the boundary between non-vascular and vascular tissues, the viruses move to other parts of the plant, resulting in systemic infection and development of viral disease. Both cell-to-cell and systemic spread of plant viruses represent the first major research focus of my laboratory.

A. Viral Cell-to-Cell Movement and Its Regulation. Cell-to-cell movement of plant viruses is mediated by a unique class of biological molecules known to specifically and dramatically alter plasmodesmal function: viral cell-to-cell movement proteins. The best characterized movement protein is the P30 polypeptide of tobacco mosaic virus (TMV) which mediates cell-to-cell spread of the viral genomic RNA. P30 interacts with plasmodesmata to increase their size exclusion limit; we have also shown that P30 cooperatively binds viral RNA and shapes it into unfolded cell-to-cell

transport complexes which can be translocated through the enlarged plasmodesmal channels. Recently, we used P30 as a molecular tool to study cellular proteins involved in plasmodesmal transport. We have isolated a host cell protein which specifically interacts P30 *in vivo* and *in vitro*. Microsequencing of the purified protein identified it as a pectin methyl esterase (PME) that binds P30 and, thus, may function as a specific P30 receptor during viral cell-to-cell movement. Supporting this idea, our results demonstrate that P30-PME interaction involves P30 domains required for its function *in vivo*.

Recent studies indicate that although P30 is present within plasmodesmata of all infected cells, it increases the plasmodesmal permeability only at the leading edge of the expanding infection site. Thus, P30 activity within cells behind the leading infection edge must be negatively regulated to prevent its continuous interference with the host plant intercellular communication. The molecular mechanism by which such regulation occurs is unknown. We addressed this question by demonstrating that P30 is phosphorylated *in vivo* at its carboxy terminus. Mimicking P30 phosphorylation by negatively charged amino acid substitutions severely disturbs P30 ability to interact with plasmodesmata and to promote viral cell-to-cell movement, suggesting that phosphorylation acts as a negative regulator of plasmodesmal transport. Interestingly, this regulatory effect on plasmodesmal permeability as well as the level of P30 phosphorylation are host dependent and may contribute to the differential susceptibility of various host plants to TMV.

In addition to understanding viral cell-to-cell transport, these experiments will identify the protein constituents of plasmodesmata, the enigmatic structures which, with their virtually unknown molecular composition, represent one of the “holy grails” of plant biology today.

B. Viral Systemic Spread. Another strategy to study plant virus movement is to isolate host mutants defective for viral spread. To achieve this, we have developed a genetic assay utilizing infection of *Arabidopsis thaliana*, a plant of choice for genetic experiments with turnip vein clearing tobamovirus (TVCV). Using this approach, we have identified an novel type of mutants (*vsm1*, virus systemic movement) in which the invading virus spreads locally within the inoculated leaf but is unable to exit this tissue and move systemically. In the absence of systemic infection, *vsm1* plants do not show symptoms of viral disease. Genetic segregation indicated that the *vsm1* phenotype is caused by a single recessive gene. Characterization of the *vsm1* plants represents the first step in genetic dissection of viral systemic movement.

To further study the systemic spread of plant viruses, we have developed specific inhibitors of this process. Exposure of tobacco plants to non-toxic concentrations of heavy metal cadmium was demonstrated to completely block viral disease caused by TVCV. Cadmium-mediated viral protection was due to inhibition of the systemic movement of the virus, *i.e*., its spread from the inoculated into uninoculated leaves. Treatment of plants with cadmium had no effect on viral replication and local movement within the inoculated leaf. Furthermore, higher, toxic levels of cadmium did not produce this inhibitory effect on viral movement, allowing the systemic spread of TVCV and development of the viral disease. These observations suggest that cadmium-induced viral protection requires a metabolically-active healthy plant. Thus, non-toxic levels of cadmium may trigger the production of cellular factors which interfere with the viral systemic movement.

Using confocal immunofluorescence microscopy, we demonstrated that, in the infected plants exposed to non-toxic levels of cadmium, TVCV virions entered the plant vascular tissue but were unable to exit it into the non-inoculated, systemic organs. This is in contrast to *vsm1* plants in which viral systemic spread was blocked at the entry into the vasculature. These results suggest that viral entry into and exit from the vascular cells occur by different mechanisms.

C. Development of Viral Disease. In addition to viral movement, my laboratory studies a still poorly understood role of host factors in development of plant viral diseases. to this end, we are isolating *Arabidopsis* mutants with alterations in the disease symptoms caused by TVCV. So far, we have identified one such mutant, *vid1* (virus-induced dwarf), which is indistinguishable from the wild-type plants when healthy but develops a severely dwarfed phenotype with a loss of apical dominance following viral infection. Genetic segregation showed that the this phenotype is caused by a recessive mutation in a single gene. The effect of *vid1* mutation was reversed by exogenous application of a plant hormone auxin. Systemic viral infection is thought to interfere

with the host plant intercellular transport; we propose that the *vid1* mutation may also affect this transport process. Combination of the mutation and viral infection may disrupt transport of developmental regulators, such as hormones, resulting in the *vid1* phenotype. The study of *vid1* plants may help to understand the involvement of hormonal responses in formation of plant viral diseases.

II. Nuclear transport of *Agrobacterium* T-DNA and viral genomes

Nucleo-cytoplasmic shuttling of macromolecules is a basic biological process central to the regulation of gene expression which underlies all aspects of development, morphogenesis, and signaling pathways in eukaryotic organisms. Furthermore, nuclear transport of proteins and protein-nucleic acid complexes is an essential step in many host-pathogen interactions such as viral and bacterial infection. These processes represent the second major research focus of my laboratory.

A. Novel Genetic Assay for Nuclear Transport. To facilitate detection of protein traffic into and out of the cell nucleus, we have developed a simple genetic assay to identify active nuclear import (NLS) and export targeting signals (NES) based on their function within yeast cells. In this system, the bacterial LexA protein was modified (mLexA) to abolish its intrinsic nuclear targeting activity and fused to the activation domain of the yeast Gal4 protein (Gal4AD) in the absence or presence of the SV40 large T-antigen NLS. In the nuclear import assay, if a protein of interest fused to the mLexA-Gal4AD hybrid contains a functional NLS, the fusion product will enter the yeast cell nucleus and activate the expression of reporter genes. In the nuclear export assay, if a protein of interest fused to the mLexA-SV40 NLS-Gal4AD hybrid contains a functional NES, the fusion product localized to the cell nucleus will exit into the cytoplasm, decreasing the reporter gene expression levels. Our results indicate that this assay may be applicable as a general method to identify and quantitatively analyze functional NLS and NES as well as specifically select for proteins containing these signals.

B. Nuclear Import of T-DNA During Genetic Transformation of Plants by *Agrobacterium*. Genetic modification of plant cells by *Agrobacterium tumefaciens* is the only known natural example of DNA transport between kingdoms. In this process, a single-stranded copy (T-strand) of bacterial transferred DNA (T-DNA) is imported into the host cell nucleus and integrated into its genome, eliciting neoplastic growths on the host plant. We utilize *Agrobacterium* infection as an experimental system to investigate the molecular mechanisms by which nucleic acid molecules are transported into the cell nucleus. Our studies have identified two *Agrobacterium* proteins, VirD2 and VirE2, which associate with the T-strand and target it to and through the nuclear pore. VirD2 is covalently attached to the 5' end of the T-strand molecule whereas VirE2 cooperatively coats the rest of the single-stranded (ss) DNA, forming the transport complex (T-complex). To better understand the structure of the T-complex, we used scanning transmission electron microscopy (STEM) to analyze VirE2-ssDNA complexes formed *in vitro*. This analysis suggested that VirE2 packages ssDNA into semi-rigid, hollow cylindrical filaments with a telephone cord-like coiled structure. The outer diameter of these complexes is too large to enter the nucleus by diffusion but is within the size exclusion limits of the active nuclear import.

We then showed that the active nuclear uptake of the T-complexes is likely mediated by both VirD2 and VirE2 proteins which were found to carry NLS signals. Importantly, while VirD2 localizes to the cell nucleus both in plant and animal systems, the nuclear targeting activity of VirE2 is plant-specific. Rearrangement of a single amino acid residue in this plant-specific NLS of VirE2 enables it to function in animal cells, such as *Drosophila* embryos and *Xenopus* oocytes, efficiently delivering DNA molecules into their nuclei.

C. Host Cell Factors Involved in T-DNA Nuclear Import. Currently, we are identifying and characterizing host plant proteins that interact with *Agrobacterium* VirD2 and VirE2. First, we have cloned an *Arabidopsis* gene coding for an NLS-binding protein which is directly involved in VirD2 nuclear import. This protein, designated AtKAP_, specifically binds VirD2 *in vivo* and *in vitro*. VirD2-AtKAP_ interaction is absolutely dependent on the carboxy terminal NLS sequence of

VirD2. The deduced amino acid sequence of AtKAP_ is homologous to yeast and animal NLS receptors belonging to the karyopherin _ family. Indeed, AtKAP_ efficiently rescues a yeast mutant defective for nuclear import. Furthermore, AtKAP_ specifically mediates transport of VirD2 into the nuclei of permeabilized yeast cells.

Next, we embarked on identification of cellular factors that recognize VirE2 and mediate its nuclear uptake. Isolation of such proteins is especially interesting because VirE2 nuclear import is plant-specific. Using a yeast two-hybrid assay, we identified two *Arabidopsis* genes whose protein products, designated E2-int1 and E2-int2, specifically interact with VirE2. Amino acid sequence analysis of the predicted protein encoded by the E2-int1 cDNA revealed a certain homology to plant but not animal or yeast proteins containing a bZIP motif. Using the described above genetic assay, E2-int1 was shown to localize to the yeast cell nucleus. When VirE2 and E2-int1 were co-expressed in yeast cells, VirE2, which alone remains cytoplasmic in yeast and animal cells, was also redirected into the cell nucleus. Furthermore, E2-int1 promoted VirE2 nuclear import in mammalian (HeLa) cells. Thus, in yeast and animal systems, E2-int1 likely acts as an adapter between the plant-specific VirE2 NLS and the cellular nuclear import machinery. Indeed, deletion of VirE2 NLS blocked the E2-int1-mediated nuclear uptake of this protein in yeast and HeLa cells. These results suggest that E2-int1 may be directly responsible for the plant-specific nuclear import of VirE2.

Amino acid sequence analysis of the second VirE2 interactor, E2-int2, identified homology to the *Rga* protein of *Drosophila*, proposed to mediate interaction between chromatin proteins and the transcriptional complex. Unlike E2-int1, E2-int2 was unable to direct VirE2 into the yeast cell nucleus. However, E2-int2 and E2-int1 interacted with each other in the two-hybrid system. It is tempting to speculate that, during T-DNA nuclear import and integration, VirE2 may function in a multiprotein complex with E2-int1 and E2-int2; in this complex, E2-int1 may lead it into the plant cell nucleus whereas E2-int2 may direct it to the future integration site in the host cell chromosome.

D. Nuclear Import and Export of Viral Genomes. In our laboratory, infection by the tomato yellow leaf curl geminivirus (TYLCV), is a major pathogen of tomato plants around the world, is used as a model system to examine nucleo-cytoplasmic shuttling of proteins and nucleic acids. Unlike most other gemini-viruses which divide their genome between two ssDNA molecules, TYLCV contains only one genomic ssDNA encapsulated by the viral capsid protein (CP). The mechanism by which TYLCV genomes enter the host cell nucleus for replication and transcription and then exit it for cell-to-cell spread is unknown.

In an international collaboration, we have shown that TYLCV CP carries a functional NLS and is likely involved in nuclear targeting of the viral genomic DNA molecules. Furthermore, our experiments using the genetic assay for protein nuclear export (see above) identified a functional NES within CP, suggesting that this protein also plays a role in exporting TYLCV genomes from the host cell nucleus. Thus, CP may represent the first plant viral structural protein implicated in both nuclear import and export of the viral genomes.

Future Research Directions

Plasmodesmata and nuclear pores are the only known membrane channels capable of trafficking large macromolecular conglomerates such as RNA- and DNA-protein complexes. Thus, I feel that my lab is uniquely positioned to investigate both types of transport through “cross-talk” between my two major projects; for example, the insights gained in the study of nuclear import or export will likely facilitate understanding the mechanisms of transport through plasmodesmata.

Ultimately, we aim to elucidate all components of cellular pathways leading to plasmodesmal and nuclear transport of viral and *Agrobacterium* proteins and nucleic acids. We have already advanced toward this goal by identifying several cellular factors that directly interact with viral P30 and *Agrobacterium* VirD2 and VirE2 proteins. Additional participants of these transport pathways will be identified in our future experiments using the, so far successful, two-prong approach: biochemical and genomic.

Genomics experiments, which are becoming the major research tool in my lab, will be performed within the framework of the functional genomics grant from NSF ($1 mil direct costs to

my lab) that I have recently received together with Stan Gelvin (Purdue Univ.) and Barbara Hohn (FMI, Basel). Below, I summarize our major research objectives for the near future.

I. Intercellular transport of plant viruses

Our first immediate goal is to isolate the cell wall-associated protein kinase responsible for regulation of the P30 activity. This enzyme will be purified biochemically, microsequenced and its encoding gene cloned, representing the first regulatory component of plasmodesmata identified to date. We then plan to use the P30 kinase as ligand in biochemical experiments or as bait in the two-hybrid approach to isolate other plasmodesmal components. Thus, in addition to understanding viral transport, this line of research will identify the protein constituents of plasmodesmata, the enigmatic structures which, with their virtually unknown molecular composition, represent one of the "holy grails" of plant biology today.

In the genetic approach, we plan to map and positionally clone the isolated *vsm1* and *vid1* mutants. Also, the genetic screen will continue to identify additional alleles and loci involved in cell-to-cell and systemic transport of viruses. To facilitate gene cloning, we shall use the existing collections of *Arabidopsis* mutants tagged by T-DNA insertional mutagenesis.

We also shall continue searching for additional plant proteins that interact with P30. By analogy to nuclear import, plasmodesmal transport may involve cytoplasmic receptors that recognize P30 and shuttle it to the plasmodesmal channel. Thus, in addition to PME identified in our research as a cell wall receptor for P30, P30 may interact with soluble cellular factors. We plan to isolate the potential cytoplasmic P30 interactors using the two-hybrid approach. Alternatively, these plant proteins will be purified by affinity chromatography on immobilized P30 columns. The biological activity of the identified proteins will be tested using microinjection and microbombardment techniques followed by confocal microscopy. In addition, we shall use reverse genetics to identify and characterize phenotypic effects of the isolated components of the plasmodesmal transport machinery.

Finally, we shall utilize the cDNA array approach to identify plant factors elicited by low concentrations of cadmium and responsible for protection from viral systemic spread and disease. These differential expression experiments will utilize DNA chips generated by Greg Martin (Cornell Univ.) and Stan Gelvin (Purdue Univ.) and will be performed under the umbrella of the NSF functional genomics program.

II. Nuclear transport of *Agrobacterium* T-DNA and geminiviruses

This project will continue characterizing the biological function of the E2-int1 and E2-int2 proteins. To this end, we shall use reverse genetics, generating transgenic plants with specific gene knock-outs. Additional cellular proteins will then be isolated using E2-int1 and E2-int2 as baits in the two hybrid approach. Also, AtKAP_ will be used as specific ligand to isolate the ß subunit of karyopherin which, by analogy to animal systems, should be required for nuclear import. Ultimately, we plan to identify and characterize all protein components of the molecular pathway leading to the T-DNA nuclear import and integration.

We shall also screen T-DNA-tagged mutants of *Arabidopsis* for alterations in T-DNA nuclear import and/or integration. This approach will be taken in collaboration with Stan Gelvin (Purdue Univ.) as one of the specific goals of our joint NSF genomics grant. In the same collaborative effort, we shall utilize cDNA arrays to identify host genes induced or repressed by *Agrobacterium* infection. My laboratory will assay their protein products for involvement in nuclear import and integration of the T-DNA.

Our studies of nuclear export will aim to precisely identify the NES signal of TYLCV CP and use it to isolate its putative cellular receptor. This latter factor will represent the first protein component of the plant nuclear export pathway.

Besides expanding our knowledge of basic cellular processes, this research has several practical implications. For example, it may develop novel approaches for production of agronomically important plants resistant to infection by viral and bacterial pathogens. Also, our work can lead to new and efficient procedures for delivery of foreign genes into the cell nucleus.

Current Grants

National Institutes of Health "Proteins Involved in Transport Through Plasmodesmata" 07/01/97 - 06/30/01

National Science Foundation, Plant Genome Research Program "Identification and Characterization of Arabidopsis Genes Involved in Interaction with Agrobacterium tumefaciens" 09/01/99 - 08/30/04, (with S. Gelvin, Purdue Univ. and B. Hohn, FMI, Basel, Switzerland),

United States Department of Agriculture "Nuclear Import of Nucleic Acid-Protein Complexes in Plants" 09/15/98 - 09/14/00

United States-Israel Binational Agricultural Research and Development Fund (BARD) "Viral and Host Cell Determinants of Nuclear Import and Export of the Tomato Yellow Curl Leaf Virus in Tomato Plants" 09/01/99 - 08/30/02, (with D. Gafni, Volcani Center)

United States-Israel Binational Science Foundation (BSF) "Plant Nuclear Import: Specificity and Role in Development and Morphogenesis", 09/01/99 - 08/30/02, (with A. Loyter, Hebrew Univ.)

New York State Science and Technology Foundation, "Protein-Facilitated Nuclear Targeting of Genes of Interest" 07/01/96 - 06/30/00

National Institutes of Health, "Biostructure Mapping by STEM, Cryo-EM, EELS and SPM; Subtitle: Structure of Protein Nucleic Acid Complexes Involved in Nuclear Import" 1996 - 2001

Postdoctoral Fellowships

United States-Israel Binational Agricultural Research and Development Fund (BARD) Postdoctoral Fellowship (to Dr. T. Tzfira), FI-269-98, "Arabidopsis Mutants with Altered Symptoms of Viral Infection" 09/01/98 - 08/30/99

United States-Israel Binational Agricultural Research and Development Fund (BARD) Postdoctoral Fellowship (to Dr. T. Kunik), FI-278-98, "Arabidopsis Mutants with Altered Symptoms of Viral Infection" 09/01/98 - 08/30/99

Fellowship from Japan Society for the Promotion of Science for Young Scientists, Science and International Affairs Bureau of Ministry of Education of Japan (to Dr. S. Ueki), 10/01/98 - 03/31/00

Past Grants

National Institutes of Health "Proteins Involved in Transport Through Plasmodesmata" 01/01/94 - 12/31/96

United States-Israel Binational Science Foundation (BSF) "DNA Translocation Across Nuclear Envelopes: Effect of Agrobacterium VirE2" 09/01/94 - 08/30/97

United States Department of Agriculture "Nuclear Import of Nucleic Acid-Protein Complexes in Plants" 09/15/94 - 09/14/96

United States-Israel Binational Agricultural Research and Development Fund (BARD) "Nuclear Import of the Tomato Yellow Curl Leaf Virus in Tomato Plants" 09/01/94 - 07/15/98

United States Department of Agriculture "Nuclear Import of Nucleic Acid-Protein Complexes in Plants" 09/15/96 - 09/14/98