18. G. G. Schaber, E. M. Shoemaker, R. C. Kozak, *Astron. Vestnik* **21**, 144 (1987).
19. The National Astronomy and Ionosphere Center is operated by Cornell University under a cooperative agreement with the National Science Foundation and with support from the National Aeronautics and Space Administration (NASA). This research was also supported by grants from the Planetary Geology and Geophysics and the Planetary Astronomy Programs of the Solar System Exploration Division of NASA to D.B.C. and J.W.H. We thank personnel at the Arecibo Observatory and students and staff in the Brown University Planetary Geology Group for assistance.

16 May 1989; accepted 28 August 1989

Movement Protein of Tobacco Mosaic Virus Modifies Plasmodesmatal Size Exclusion Limit

SHMUEL WOLF, CARL M. DEOM, ROGER N. BEACHY, WILLIAM J. LUCAS

The function of the 30-kilodalton movement protein (MP) of tobacco mosaic virus is to facilitate cell-to-cell movement of viral progeny in an infected plant. A novel method for delivering non–plasmalemma-permeable fluorescent probes to the cytosol of spongy mesophyll cells of tobacco leaves was used to study plasmodesmatal size exclusion limits in transgenic plants that express the MP gene. Movement of fluorescein isothiocyanate–labeled dextran (F-dextran) with an average molecular mass of 9400 daltons and an approximate Stokes radius of 2.4 nanometers was detected between cells of the transgenic plants, whereas the size exclusion limit for the control plants was 700 to 800 daltons. No evidence of F-dextran metabolism in the leaves of the transgenic plants was found. Thus, the tobacco mosaic virus movement protein has a direct effect on a plasmodesmatal function.

PLASMODESMATA ARE NARROW strands of cytoplasm that penetrate adjoining cell walls to interconnect plant cells, thus forming a community of living protoplasts termed the symplasm. Cells and tissues that are remote from direct sources of nutrients can be nourished by the movement of carbohydrates, amino acids, and inorganic ions through plasmodesmata. Plasmodesmata also represent potential pathways for the passage of signals, either electrical or hormonal, which could integrate and regulate the activities of different parts of the symplasm (*1*).

The function of the symplasmic pathway in tissues and organs of diverse plant species has been reported (*2*). Techniques for microinjection of nontoxic membrane-impermeable fluorescent dyes (for example, Lucifer yellow CH), used extensively for tracing neurological interactions (*3*) and studying gap junctions (*4*), have been used in a number of plant tissues (*2, 5–7*). Synthesis of fluorescent peptide probes of known molecular mass and radius, developed to probe the size exclusion limits of gap junctions (*8*), has also been exploited by plant scientists to establish the extent of symplasmic permeability in plant tissues (*2, 5–9*).

Certain types of plant viruses spread throughout the host by moving through plasmodesmata, as in the case of mosaic viruses (*10–12*). Electron microscopic evidence of viral particles moving through plasmodesmata of a variety of plants has been reported (*11, 13*). Virus gene expression, therefore, may provide a system for studying plasmodesmata as well as virus movement (*14, 15*).

Conclusive evidence that the 30-kD movement protein (MP) of tobacco mosaic virus (TMV) is involved in cell-to-cell movement of the virus was demonstrated by Deom *et al.* (*14*). Expression of the MP gene in transgenic plants complemented the Ls1 mutant of TMV, a mutant that is temperature-sensitive (ts) in cell-to-cell movement (*16*). In transgenic plants infected with Ls1 and maintained at the nonpermissive temperature, cell-to-cell movement of the Ls1 virus was potentiated in both inoculated and upper systemic leaves. Although this finding provides direct evidence that the MP of TMV is necessary for virus movement, little is known about the mode by which the MP facilitates movement. We now show that the expression of the TMV MP gene in trans-

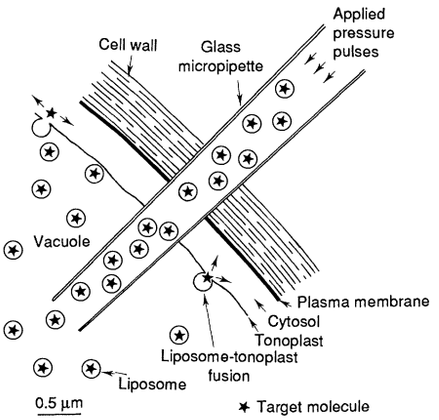

Fig. 1. Schematic representation of the technique employed to introduce fluorescently labeled target molecules, in vivo, into the cytosol of tobacco mesophyll cells. Lucifer yellow CH (LYCH; Sigma) and FITC-labeled hexaglycine ($F\text{-}Gly_6$) and dextrans with molecular masses of 3,900, 9,400, and 17,200 daltons were used as fluorescent probes and were prepared as described by Simpson (*17*). Liposomes were prepared by the freeze-thaw method described by Pick (*18*) as modified by Madore *et al.* (*5*) and were back-loaded via capillary action into the tips of glass micropipettes having a tip diameter of 0.5 to 1.0 μm. The capillary was sealed into a micropipette holder equipped with a luer port, and pressure was controlled by a Pneumatic PicoPump (World Precision Instruments, model PV830). Injection pressure was 7 to 15 psi. Pipette movement for cell impalement was controlled by a hydraulically driven micromanipulator (Narishige model MO-102).

Table 1. Mobility of fluorescent probes through the symplasmic pathway of mesophyll cells of transformed tobacco plants. Data are presented as the percentage of injections that showed movement of the specific probe, as determined 2 min after injection. (Values in parentheses represent number of injections).

Probe*	Molecular mass (daltons)	Transgenic plant line	MP genotype	Percentage of injections expressing movement
LYCH	457	277	MP^+	100 (5)
		306	MP^-	100 (5)
$F\text{-}Gly_6$	749	277	MP^+	100 (8)
		306	MP^-	50 (10)
F-Dextran	3,900	277	MP^+	100 (6)
		306	MP^-	14 (7)
F-Dextran	9,400	277	MP^+	93 (15)
		306	MP^-	0 (12)
F-Dextran	17,200	277	MP^+	0 (6)
		306	MP^-	0 (6)

*Described in Fig. 1.

S. Wolf and W. J. Lucas, Botany Department, University of California, Davis, CA 95616.
C. M. Deom and R. N. Beachy, Department of Biology, Washington University, St. Louis, MO 63130.

genic plants can alter the size exclusion limit of plasmodesmata.

To measure the movement of molecules between mesophyll cells, we developed a system that allowed injection of fluorescein isothiocyanate (FITC)–labeled dextrans (F-dextrans) into mesophyll cells located on the lower surface of tobacco leaves attached to plants. A mature tobacco leaf was placed, abaxial side up, on a microscope slide and held in place with a piece of double-sided transparent tape. A small portion (5 mm^2) of the lower epidermis was removed with forceps and a small well, formed by modeling clay positioned around the exposed area, was filled with glass-distilled water. An exposed spongy mesophyll cell was then impaled with a micropipette, and a suspension of liposomes that encapsulated the FITC-labeled molecules was pressure-injected into the vacuole (Fig. 1).

Movement of the dye was monitored with a Leitz Orthoplan epifluorescence microscope equipped with a blue (BP 390 to 490) excitation filter, and quantitation of dye movement was monitored with a Hamamatsu image analysis system (model C1966-20).

Probes of different molecular masses were injected into leaf mesophyll cells of plant lines 306 and 277 (*19*) and observed by fluorescence microscopy. Molecules of less than 749 daltons moved with equal efficiency in lines 306 and 277 (Table 1). F-Dextran of 3900 daltons or greater did not move from injected cells in plant line 306 even after 20 min (Table 1 and Fig. 2F). By contrast, after injection of F-dextrans of 3900 daltons into cells of line 277, the molecules had moved from the injected cells in 100% of the experiments (Table 1). When F-dextrans of 9400 daltons were injected, the molecules had moved, after 15 s, from injected cells in 93% of the experiments (Table 1 and Fig. 2B). By 30 to 60 s after injection, traces of the larger dextran were found four to five cells from the injection site, which represents a distance of 150 to 200 μm (Fig. 2C). No movement of F-dextran of 17,200 daltons was observed in either plant line within 20 min after injection.

We conducted an experiment to determine whether or not there was specific degradation of the higher molecular mass F-dextran in plant line 277 that could account for the observed fluorescence pattern. Leaf extracts from plant line 277 that had been injected with F-dextran of 9400 daltons were separated by paper chromatography and analyzed by fluorescence detection with our Leitz Orthoplan–Hamamatsu Analytical Photon Counting system. No evidence of degraded FITC probe was found, which led us to conclude that the F-dextran probes were not metabolized by the mesophyll cells of the tobacco plant line 277 (*20*).

Studies on cell-to-cell movement in plants indicate that the molecular exclusion limits of plasmodesmata fall within 700 to 800 daltons (*2, 6, 21*). We found similar size limits in movement between spongy mesophyll cells of our control plants (line 306). By contrast, plant line 277, which expresses the TMV MP gene, permitted movement of molecules with a molecular mass more than ten times as great (9400 daltons). Thus a TMV protein can alter plasmodesmatal function.

The mechanism (or mechanisms) by which the TMV MP (or proteins with similar function from other viruses) might function to modify plasmodesmata is unknown. It is generally accepted that viruses or their nucleic acids can move through plasmodesmata, but their possible effect on these structures is conjectural. Atabekov and Dorokhov (*22*) proposed a possible mechanism for cell-to-cell movement involving a putative transport protein (probably the MP in the case of TMV) that "opens the gate," resulting in passage of the virus into adjacent healthy cells. Plasmodesmata could constitute such "gates" that must be modified by the viral MP for efficient virus movement.

A preliminary electron microscopy study on plant lines 277 and 306 showed no significant difference between their plasmodesmata (*23*). The axial component (desmotubule) and cytoplasmic annulus were present in both transgenic lines, indicating that a minor change in plasmodesmatal substructure, not easily detected with routine electron microscopic methods, resulted in the observed change in intercellular movement of macromolecules.

TMV particles are 18 by 300 nm, whereas the size and conformation of TMV RNA is unknown. Whether TMV particles or TMV RNA moves between mesophyll cells that are modified by the MP during infection is also unknown. The conformation in the cytoplasm of dextrans, or F-dextrans, will probably be a random coil and, as such, the

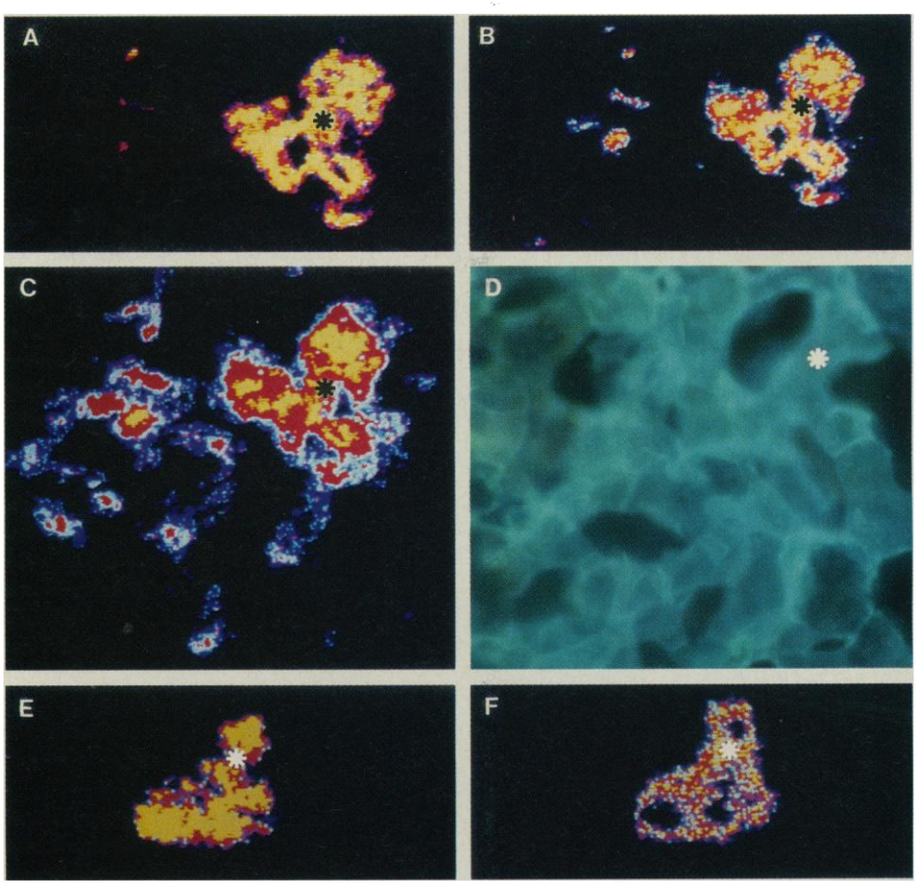

Fig. 2. Movement of F-dextran in tobacco leaf cells after microinjection of liposome-encapsulated dye into a spongy mesophyll cell, as indicated by false-color imaging obtained with an Hamamatsu model C1966-20 analytical system. Movement of F-dextran of 9400 daltons in the leaf of the transgenic plant (277) (**A**) 5 s, (**B**) 15 s, and (**C**) 60 s after injection. (**D**) Fluorescent image of the region depicted in (A) to (C) after staining with Calcofluor White to aid visualization of individual cell walls (×300). Containment of 3900-dalton F-dextran within the injected mesophyll cell of the control plant (line 306) is evident (**E**) 2 min and (**F**) 20 min after injection (×600).

"effective" Stokes radius can be calculated or determined empirically (*24*). A dextran polymer of 10,000 daltons would have an effective Stokes radius of 2.4 nm, whereas the radius of an 18,000-dalton dextran polymer would be approximately 3.1 nm. On this basis, the molecular size exclusion limit (Stokes radius) of a plasmodesma in control tobacco plants would be approximately 0.73 nm, whereas that of plasmodesmata in plants that express the 30-kD MP would be greater than 2.4 nm but less than 3.1 nm. Since Gibbs (*12*) has suggested that the molecular dimensions of TMV RNA may be approximately 10 nm, additional information is needed before we can account for the role of the MP in mediating systemic spread of TMV.

REFERENCES AND NOTES

1. B. E. S. Gunning, in *Intercellular Communication in Plants: Studies on Plasmodesmata*, B. E. S. Gunning and A. W. Robards, Eds. (Springer-Verlag, Berlin, 1976), pp. 1–13; ——— and A. W. Robards, in *Transport and Transfer Processes in Plants*, I. F. Wardlaw and J. B. Passioura, Eds. (Academic Press, New York, 1976), pp. 15–41.
2. M. G. Erwee and P. B. Goodwin, *Planta* **158**, 320 (1983); *Protoplasma* **122**, 162 (1984); ———, A. J. E. van Bel, *Plant Cell Environ.* **8**, 173 (1985); M. G. Erwee and P. B. Goodwin, *Planta* **163**, 9 (1985); D. G. Fisher, *Plant Cell Environ.* **11**, 639 (1988); K. J. Oparka and D. A. M. Prior, *Planta* **176**, 533 (1988).
3. W. W. Stewart, *Nature* **292**, 17 (1981).
4. W. R. Loewenstein and B. Rose, *Ann. NY Acad. Sci.* **307**, 285 (1978).
5. M. A. Madore, J. W. Oross, W. J. Lucas, *Plant Physiol.* **82**, 432 (1986).
6. E. B. Tucker, *Protoplasma* **113**, 193 (1982).
7. B. A. Palevitz and P. K. Helper, *Planta* **164**, 473 (1985).
8. J. Flagg-Newton, I. Simpson, W. R. Loewenstein, *Science* **205**, 404 (1979).
9. B. R. Terry and A. W. Robards, *Planta* **171**, 145 (1987).
10. K. Esau, Ed., *The Phloem: Handbuch der Pflanzenanatomie* (Gebrueder Borntraeger, Berlin, 1969).
11. E. W. Kitajima and J. A. Lauritis, *Virology* **37**, 681 (1969).
12. A. Gibbs, in *Intercellular Communication in Plants: Studies on Plasmodesmata*, B. E. S. Gunning and A. W. Robards, Eds. (Springer-Verlag, Berlin, 1976), pp. 149–164.
13. A. V. Allison and T. A. Shalla, *Phytopathology* **64**, 784 (1974); M. Weintraub, H. W. J. Ragetli, E. Leung, *J. Ultrastruct. Res.* **56**, 351 (1976).
14. C. M. Deom, M. J. Oliver, R. N. Beachy, *Science* **237**, 389 (1987).
15. T. Meshi *et al.*, *EMBO J.* 6, 2557 (1987).
16. M. Nishiguchi, F. Motoyoshi, N. Oshima, *J. Gen. Virol.* **39**, 53 (1978); *ibid.* **46**, 497 (1980).
17. I. Simpson, *Anal. Biochem.* **89**, 304 (1978).
18. U. Pick, *Arch. Biochem. Biophys.* **212**, 186 (1981).
19. Transgenic tobacco plant line 277 (expressing the 30-kD protein) and line 306 (harboring the intermediate plasmid only) were used for all experiments. Seeds were germinated and plants were grown for 3 weeks in an insect-free greenhouse and then transferred to a growth chamber for 1 to 2 weeks before the measurements. Temperature regime in the growth chamber was 27°/18°C (day/night) with 16 hours of light.
20. S. Wolf and W. J. Lucas, unpublished results.
21. E. B. Tucker and R. M. Spanswick, *Protoplasma* **128**, 167 (1985).
22. J. G. Atabekov and Y. L. Dorokhov, *Adv. Virus Res.* **29**, 313 (1984).
23. W. J. Lucas and T. C. Pesacreta, unpublished results.
24. T. C. Laurent and J. Killander, *J. Chromatogr.* **14**, 317 (1964); R. H. Pearce and B. J. Grimmer, *ibid.* **151**, 548 (1978); P. G. Squire, *ibid.* **210**, 433 (1981); E. Poitevin and P. Wahl, *Biophys. Chem.* **31**, 247 (1988).

25. Supported by grants from the National Science Foundation to R.N.B. (DMB 87017012) and W.J.L. (DMB 8703624).

12 June 1989; accepted 5 September 1989

The Gene for Enhancer Binding Proteins E12/E47 Lies at the t(1;19) Breakpoint in Acute Leukemias

JULIA D. MELLENTIN, CORNELIS MURRE, TIMOTHY A. DONLON, PATRICK S. MCCAW, STEPHEN D. SMITH, ANDREW J. CARROLL, MARCY E. MCDONALD, DAVID BALTIMORE, MICHAEL L. CLEARY*

The gene (*E2A*) that codes for proteins with the properties of immunoglobulin enhancer binding factors E12/E47 was mapped to chromosome region 19p13.2–p13.3, a site associated with nonrandom translocations in acute lymphoblastic leukemias. The majority of t(1;19)(q23;p13)–carrying leukemias and cell lines studied contained rearrangements of *E2A* as determined by DNA blot analyses. The rearrangements altered the *E2A* transcriptional unit, resulting in the synthesis of a transcript larger than the normal-sized *E2A* mRNAs in one of the cell lines with this translocation. These observations indicate that the gene for a transcription factor is located at the breakpoint of a consistently recurring chromosomal translocation in many acute leukemias and suggest a direct role for alteration of such factors in the pathogenesis of some malignancies.

CHROMOSOMAL TRANSLOCATIONS are implicated as important events in the pathogenesis of tumors of the hematopoietic lineage. The majority of acute lymphoblastic leukemias (ALLs) carry such karyotypic abnormalities, although more than 15 different consistently recurring rearrangements have been described (*1*). One of the most frequently reported cytogenetic changes in ALL is the t(1;19)(q23;p13.3) chromosomal translocation, first reported in 1984 and subsequently observed in up to 6% of pediatric acute leukemias and in approximately 30% of leukemias with a pre-B cell phenotype (*2*). The association of this translocation with a particular leukemia phenotype has prompted investigations into the identity of genes that may map to the breakpoint loci on chromosomes 1 and 19 (*3*). Despite suggestive chromosomal localizations, neither the insulin receptor gene at 19p13.2–p13.3 nor the proto-oncogene c-*ski* at 1q22–24 has been shown to map to the breakpoint in the t(1;19) translocation. We now report that the gene for a transcription factor that appears to regulate immunoglobulin kappa light chain gene expression is located at the breakpoint on chromosome 19 and, furthermore, we demonstrate that this gene is structurally altered by most t(1;19) chromosomal translocations.

Various trans-acting factors and sequence motifs required for tissue-specific expression of the immunoglobulin genes have been described (*4*). One class of such sequences is the "E-box motif," found in both the heavy chain and kappa light chain enhancers (*5–7*); trans-acting factors that interact with the E-box sites both in vivo and in vitro have been identified (*5, 6*). In the enhancer region of the kappa light chain gene, E-box elements are required for optimal transcription and have been shown to bind distinct proteins in vitro (*6, 7*). Two cDNAs (*E12/E47*) that encode proteins that bind to the kappa E-box site kE2 have been isolated by an oligonucleotide screening procedure (*8*) and have been found to be derived from one gene, which has been called *E2A* (*9*). The cDNAs code for nearly identical proteins, which contain a region that appears to represent a helix-loop-helix DNA binding motif and a dimerization domain. This region is similar to regions in several proteins that participate in the control of differentiation and prolif-

J. D. Mellentin, T. A. Donlon, M. L. Cleary, Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94025.
C. Murre, P. S. McCaw, D. Baltimore, Whitehead Institute for Biomedical Research, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
S. D. Smith, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94025.
A. J. Carroll, Laboratory of Medical Genetics, University of Alabama at Birmingham, Birmingham, AL 35294, and Pediatric Oncology Group, St. Louis, MO 63108.
M. E. McDonald, Neurogenetics Laboratory, Massachusetts General Hospital, Boston, MA 02114, and Department of Genetics, Harvard Medical School, Boston, MA 02115.

*To whom correspondence should be addressed.