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Movement Protein of Tobacco Mosaic Virus Modifies Plasmodesmatal Size Exclusion Limit

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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-

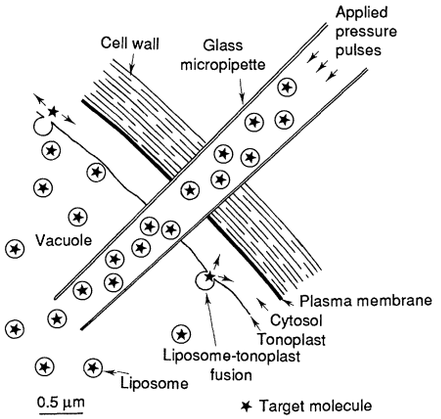

Fig. 1. Schematic representation of the technique employed to introduce fluorescently labeled target molecules, in vivo, into the cytosol of tobacco mesophyll cells. Lucifer yellow CH (LYCH; Sigma) and FITC-labeled hexaglycine (F-Gly_6) and dextrans with molecular masses of 3,900, 9,400, and 17,200 daltons 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-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.

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