was least able to transport itself, did not potentiate the cell-to-cell transport of *kn1* sense RNA-TOTO (Table 3).

KN1 was selective in terms of the RNA that it would traffic, as shown by coinjection of TOTO-labeled cucumber mosaic virus (CMV) single-stranded sense RNA (*18*) and KN1 (Fig. 4C, Table 3). The CMV movement protein, in contrast, potentiated cell-to-cell transport both of its own RNA and of *kn1* RNA (Fig. 4D, Table 3), which is consistent with the known nonspecificity of viral movement proteins (*6*–*8*).

Our finding that KN1 has the capacity to move from cell to cell provides a possible explanation for the lack of cell autonomy seen with the dominant *Kn1* mutation as well as with other developmental mutations (*3*, *4*, *19*). How such plasmodesmal transport is controlled to create developmental domains (*5*) remains to be elucidated. The extent to which a transcription factor can move within a tissue may be controlled by the presence of proteins that regulate its plasmodesmal and nuclear pore transport. This might explain why, in the maize meristem, KN1 was present in both L1 and L2 nuclei (Fig. 1), whereas in tobacco mesophyll cells, microinjected FITC-KN1 moved preferentially through plasmodesmata rather than into nuclei.

In any event, our studies on KN1 provide insights into some of the molecular events that orchestrate developmental processes in plants and identify one possible explanation for the plasticity of cell fate in the plant meristem (*2*).

REFERENCES AND NOTES

1. E. Huala and I. M. Sussex, *Plant Cell* **5**, 1157 (1993); I. M. Sussex, *Cell* **56**, 225 (1989).
2. W. J. Lucas, *Curr. Opin. Cell Biol.* **7**, 673 (1995).
3. R. Carpenter and E. S. Coen, *Development* **121**, 19 (1995); S. S. Hantke, R. Carpenter, E. S. Coen, *ibid.*, p. 27.
4. E. J. Symkowiak and I. M. Sussex, *Plant Cell* **4**, 1089 (1992).
5. W. J. Lucas, B. Ding, C. Van der Schoot, *New Phytol.* **125**, 435 (1993); W. J. Lucas and S. Wolf, *Trends Cell Biol.* **3**, 308 (1993).
6. W. J. Lucas and R. L. Gilbertson, *Annu. Rev. Phytopathol.* **32**, 387 (1994).
7. T. Fujiwara, D. Giesman-Cookmeyer, B. Ding, S. A. Lommel, W. J. Lucas, *Plant Cell* **5**, 1783 (1993); A. O. Noueiry, W. J. Lucas, R. L. Gilbertson, *Cell* **76**, 925 (1994); E. Waigmann, W. J. Lucas, V. Citovsky, P. Zambryski, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1433 (1994).
8. B. Ding, L. Qiubo, L. Nguyen, P. Palukaitis, W. J. Lucas, *Virology* **207**, 345 (1995).
9. S. Hake and M. Freeling, *Nature* **320**, 621 (1986); N. Sinha and S. Hake, *Dev. Biol.* **141**, 203 (1990); E. Vollbrecht, B. Veit, N. Sinha, S. Hake, *Nature* **350**, 241 (1991); L. G. Smith, B. Greene, B. Veit, S. Hake, *Development* **116**, 21 (1992).
10. In situ hybridization and immunolocalization experiments were performed on paraffin-embedded maize seedling apices. In situ hybridization was performed exactly as described (*11*), whereas for immunolocalization we used the method of Smith *et al.* (*9*), except that tissue was embedded in paraffin wax and sections were predigested with proteinase K (Sigma) at 100 μg/ml in phosphate-buffered saline (PBS) for 10 min at room temperature and then rinsed twice in PBS before the blocking step. Goat antibody to rabbit alkaline phosphatase (Boehringer Mannheim) was used as the secondary antibody (1:600 dilution) and visualized according to the method of Jackson *et al.* (*11*). Sections were lightly counterstained in basic fuchsin (0.005% w/v).
11. D. Jackson, B. Veit, S. Hake, *Development* **120**, 405 (1994).
12. Wild-type and mutant KN1 were expressed, extracted, and labeled with FITC according to the procedures we developed for viral movement proteins (*7*, *8*). As an internal control, proteins were extracted and FITC-labeled from an *E. coli* preparation that did not contain the *kn1* complementary DNA (cDNA). Alanine scanning mutants were created in groups of charged amino acids, which are likely to be present in surface domains (PC gene software, Intelligenetics). The *kn1* cDNA (Bam HI–Nco I partial digest) from pKOC10 was inserted into the pET23-d(+) vector (Novagen) to create pDJX-1. Single-stranded virions were produced in the CJ236 (*dut ung*) strain of *E. coli*, and site-directed mutagenesis was performed with oligonucleotides of 33 to 48 bases and with T7 DNA polymerase, according to the manufacturer's instructions (U.S. Biochemical). Mutagenized clones were confirmed by being sequenced before transfer to strain BL21(DE3) for protein production.
13. Microinjections were carried out essentially as previously described [S. Wolf, C. M. Deom, R. N. Beachy, W. J. Lucas, *Science* **246**, 377 (1989)], except for the modifications noted in (*7*, *8*).
14. N. Sinha, R. Williams, S. Hake, *Genes Dev.* **7**, 787 (1993).
15. A. W. Robards and W. J. Lucas, *Annu. Rev. Plant Physiol. Plant Molec. Biol.* **41**, 369 (1990).
16. E. Vollbrecht, R. Kerstetter, B. Lowe, B. Veit, S. Hake, in *Evolutionary Conservation of Developmental Mechanisms*, A. C. Spalding, Ed. (Wiley-Liss, New York, 1993), pp. 111–123; R. Kerstetter *et al.*, *Plant Cell* **6**, 1877 (1994); C. Lincoln, J. Long, J. Yamaguchi, K. Serikawa, S. Hake, *ibid.*, p. 1859.
17. *Kn1* sense or antisense RNA was transcribed with the use of T3 or T7 RNA polymerase from linearized pKOC10 plasmid that contained the full-length cDNA. The DNA template was digested with RQ1 DNase (Promega), and the RNA was phenol-extracted and ethanol-precipitated. *Kn1* RNA (1.6 kb) was resuspended in 20 μl of diethyl pyrocarbonate–H_2O, and concentration and purity were determined by spectroscopy. Sense and antisense RNA (500 μg/ml) were labeled with the nucleotide-specific fluorescent probe TOTO-1 (Molecular Probes) as previously described (*7*, *8*). All *kn1* RNA-TOTO preparations were adjusted to 225 μg/ml for use in microinjection experiments. CMV RNA-TOTO was adjusted to 250 to 500 μg/ml.
18. Purified CMV RNA was prepared [P. Palukaitis and M. Zaitlin, *Virology* **132**, 426 (1984)] and TOTO-labeled as described by Ding *et al.* (*8*). This preparation contained three single-stranded RNA species, RNA1 (3.3 kb), RNA2 (3.0 kb), and RNA3 (2.2 kb). The procedures of Ding *et al.* (*8*) were used to prepare and FITC-label the CMV 3a movement protein.
19. P. W. Becraft and M. Freeling, *Genetics* **136**, 295 (1994).
20. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Interaction of Tobamovirus Movement Proteins with the Plant Cytoskeleton

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The movement protein of tobacco mosaic tobamovirus and related viruses is essential for the cell-to-cell spread of infection and, in part, determines the host range of the virus. Movement protein (MP) was fused with the jellyfish green fluorescent protein (GFP), and a modified virus that contained this MP:GFP fusion protein retained infectivity. In protoplasts and leaf tissues, the MP:GFP fusion protein was detected as long filaments shortly after infection. Double-labeling fluorescence microscopy suggests that the MP interacts and coaligns with microtubules. The distribution of the MP is disrupted by treatments that disrupt microtubules, but not by cytochalasin B, which disrupts filamentous F-actin. Microtubules may target the MP to plasmodesmata, the intercellular channels that connect adjacent cells.

Most, if not all, plant viruses direct the synthesis of one or more MPs required for the spread of infection from the initial site of infection to adjacent cells. It is generally thought that plant viruses circumvent the cell wall by exploiting plasmodesmata, specialized gatable channels that provide continuity between the cytoplasm of contiguous cells (*1*).

The most thoroughly studied virus-encoded MP is that of tobacco mosaic virus (TMV) (*2*, *3*). In plants infected with TMV or transgenically expressing MP, the MP is associated with plasmodesmata and increases their size exclusion limit (*4*, *5*). MP is targeted to the cell wall but is also found associated with the plasma membrane and

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in a soluble form (*4*, *6*), suggesting that MP might also interact with other cellular components in addition to plasmodesmata.

To determine how the MP is targeted to plasmodesmata and to identify host factors that interact with MP, we used mutants of TMV (TMV-M:Gfus) and the related tobamovirus Ob (*7*) (Ob-M:Gfus) that encode MP as a fusion to the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* (*8*). Modified viruses TMVΔC-GFP and ObΔC-GFP lead to production of GFP as a free protein. BY-2 tobacco protoplasts were inoculated by electroporation of infectious RNA (*9*) that was transcribed in vitro from cloned complementary DNAs (cDNAs) of each of the modified viruses (*10*), and the pattern of GFP fluorescence in the infected cells was examined at 16 hours post infection (hpi) (*11*).

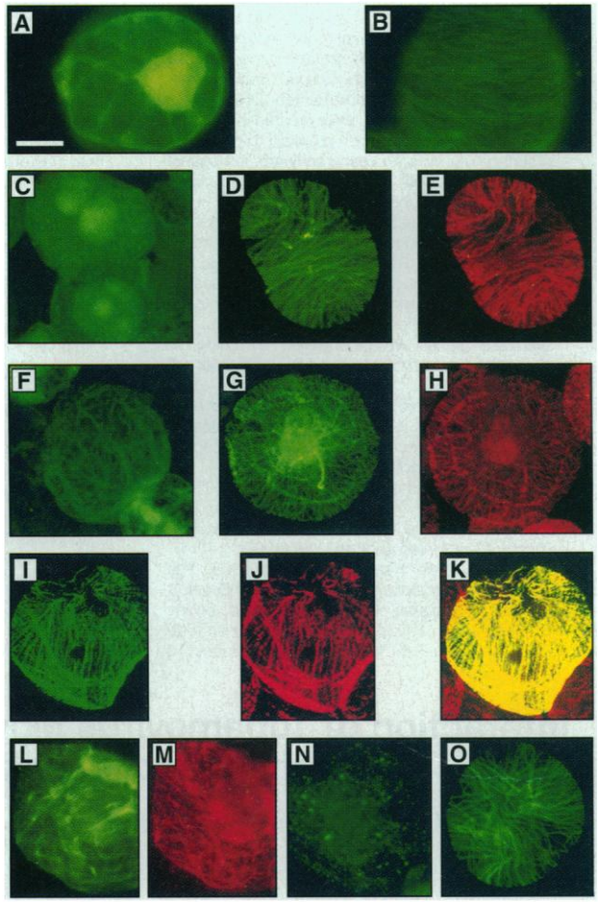

Fig. 1. Fluorescence microscopy of living (A and B) and fixed (*12*) (C to O) BY-2 protoplasts at 16 hpi. (**A** and **C**) Protoplast infected with ObΔC-GFP encoding GFP as a free protein. The same distribution of nonlocalized GFP fluorescence is seen in protoplasts infected with TMVΔC-GFP (*14*). In protoplasts infected with Ob-M:Gfus (**B**) or TMV-M:Gfus (**D**), the GFP appears as hoops of fluorescent filaments located in the cortical region of the cells. Decoration of GFP-associated filaments (D) with anti-MP (**E**). (**F**) Protoplasts infected with wild-type TMV and stained with anti-MP and then with FITC-conjugated secondary antibody. (**G** and **H**) Protoplasts infected with TMV-M:Gfus stained with monoclonal antibody against α-tubulin and rhodamine-conjugated secondary antibody. The MP:GFP filaments shown in (G) co-align with microtubules shown in (H). (**I** to **K**) Protoplasts infected with Ob-M:Gfus and observed by confocal laser-scanning microscopy: (I) the filamentous pattern of MP:GFP, (J) the pattern of microtubules, and (K) the alignment of MP:GFP filaments to microtubules visualized by merging the images shown in (I) and (J). The combination of red and green signals produces a yellow signal. (**L** and **M**) Protoplasts infected with Ob-M:Gfus probed with anti-actin and rhodamine-conjugated secondary antibody. The distribution of MP:GFP filaments shown in (L) differs from the distribution of F-actin filaments shown in (M). (**N** and **O**) MP:GFP-associated filaments in protoplasts infected with Ob-M:Gfus or TMV-M:Gfus are destroyed after treatment with oryzalin (10 μM) (N), which disrupts microtubules, but not after treatment with cytochalasin B (25 μg/ml) (O), which disrupts F-actin. Scale bar in (A) represents 20 μm; all panels are the same magnification as (A).

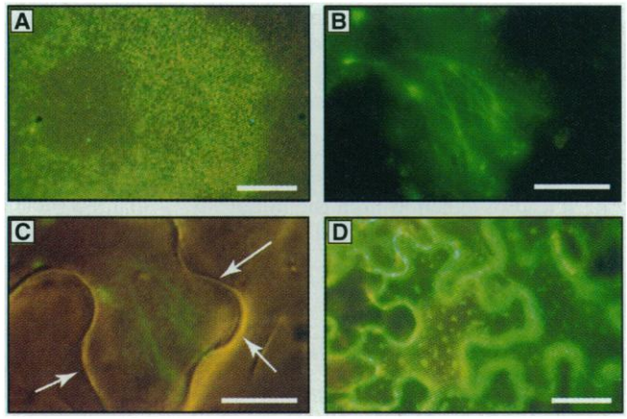

Fig. 2. Leaves of *N. benthamiana* infected with TMV-M:Gfus at 96 hpi exhibit rings of GFP fluorescence (**A**), which represent expanding sites of infection. Scale bar in (A) represents 1 mm. (**B**) Fluorescent filaments in some of the cells comprising the fluorescent ring; part of an epidermal cell is shown. (**C**) The same cell as in (B) with the cell wall (arrows) highlighted by superimposed differential interference contrast illumination. Scale bar in (B) and (C) is 50 μm. (**D**) Within the cells of the fluorescent infection site that contain the highest amounts of MP:GFP, the fusion protein appears to be localized in cytoplasmic bodies closely appressed to the plasma membrane. Scale bar represents 0.1 mm.

Protoplasts that were infected with TMVΔC-GFP or ObΔC-GFP exhibited diffuse fluorescence (Fig. 1A). In contrast, in cells that were infected with either Ob-M:Gfus or TMV-M:Gfus the GFP appeared as long, often aligned filaments that spanned the cells (Fig. 1B). The filaments were localized to the cortical region of the cells, in close proximity to the plasma membrane.

After formaldehyde fixation, the filamentous pattern of MP:GFP fluorescence was maintained (Fig. 1D). Immunostaining (*12*) of fixed cells infected by TMV-M:Gfus with an antibody to MP (anti-MP) (*2*) and a rhodamine-labeled secondary antibody (*13*) resulted in specific labeling of the GFP-containing filaments (Fig. 1E). No staining was observed in mock-infected cells (*14*). Therefore, the filaments contain MP:GFP fusion protein, and because nonfused GFP does not appear as filaments (Fig. 1, A and C), we conclude that MP is required for filament association.

A filamentous staining pattern was also observed when protoplasts infected with wild-type TMV were similarly immunolabeled with anti-MP and fluorescein isothiocyanate (FITC)–conjugated secondary antibody (Fig. 1F), suggesting that the fluorescent filaments seen in both live and fixed protoplasts infected with Ob-M:Gfus or TMV-M:Gfus reflect a distribution of the wild-type MP.

The pattern of GFP fluorescence shown in Fig. 1, B and D, is reminiscent of the cortical array of plant microtubules observed by immunofluorescent labeling (*15*, *16*). In TMV-M:Gfus– and Ob-M:Gfus–infected protoplasts stained with monoclonal antibodies to tubulin (Fig. 1, G and H), the pattern of microtubules appears to be similar if not identical to the pattern of MP:GFP filaments. Overlaying red tubulin and green MP:GFP fluorescent images made from the same cells by confocal laser scanning microscopy shows that MP:GFP filaments and microtubules are coaligned (Fig. 1, I, J, and K). In contrast, no alignment of MP to filamentous F-actin was evident after infected cells were probed with monoclonal antibodies to actin (Fig. 1, L and M) (*17*).

Infected protoplasts were also treated for 3 hours with microtubule disruptive agents before fixation and reaction with anti-tubulin. Treatments with cold temperature (4°C) (*14*), 10 μM oryzalin (Fig. 1N), or 10

μM propyzamide (*14*) disrupted MP:GFP filaments, whereas no effect was seen after treatment with the microtubule-stabilizing agent Taxol (20 μg/ml) (*14*) or with cytochalasin B (25 μg/ml), which disrupts microfilaments (F-actin) (Fig. 1O). Cells infected with TMV-M:Gfus or Ob-M:Gfus responded similarly to these treatments. We conclude that the MP filaments we observed are caused by a direct or indirect interaction of the MP with microtubules, but not with actin filaments.

To determine whether filamentous structures could be observed in planta, we inoculated leaves of *Nicotiana benthamiana* with transcripts derived from pTMV-M:Gfus. At 96 hpi, rings of fluorescence (Fig. 2A) representing expanding sites of infection were observed. Cells from the fluorescent ring contained fluorescent filaments reminiscent of those observed in infected protoplasts (Fig. 2, B and C), suggesting that the interaction between MP and microtubules occurs during virus infection of leaf tissues as well as in protoplasts. In cells adjacent to those containing fluorescent filaments we observed fluorescent bodies of irregular shape and distribution that were near the plasma membrane (Fig. 2D). On the basis of these observations we propose that the pathway of targeting MP to the plasmodesmata involves association of the protein with cortical microtubules as well as with a network of cortical bodies of unknown identity. It has been shown that both the plasma membrane and cortical endoplasmic reticulum (ER) are integral components of the plasmodesmata (*18*), and we suggest that nearby cortical microtubules (*16, 19, 20*) might provide a track for MP to reach either or both of these membranes.

Such a role of microtubules in viral infection and the targeting of MP to plasmodesmata would be consistent with their general role in cellular transport processes that contributes to the organization and distribution of organelles and their transport intermediates (*21*) and would also be consistent with several virus-microtubule interactions reported in animal cells (*22*). Moreover, the 65-kD protein of beet yellows closterovirus binds to microtubules in vitro, and it was proposed that this binding may be linked with the process of cell-to-cell movement of the virus (*23*).

Further studies are needed to clarify the nature of the MP-microtubule interaction. It is possible that MP interacts directly with cortical microtubules through binding to the outer wall of the microtubule polymer, as has been described for microtubule-associated proteins. However, because microtubules are closely associated with ER membranes in animal cells (*24*) as well as in plant cells (*25*), it is also possible that MP participates in the secretory pathway and is processed and transported within ER tubules or ER vesicles along microtubules. In plant cells ER membranes form a contiguous system with the cortical ER (*20, 26*), which as an integral component of the plasmodesmata could deliver the MP into adjacent cells.

The MP of TMV not only accumulates in plasmodesmata and modifies their size exclusion limit, but also forms unfolded and elongated complexes with single-stranded nucleic acids in vitro (*27*). This has led to the model that MP forms a ribonucleoprotein complex with the viral RNA that is compatible in size with the modified plasmodesmata. Microtubules have been implicated in transport and localization of mRNA (*28*), and the hypothesis has gained strong support by Ainger *et al.* (*29*) who observed mRNA particles moving along microtubules in microinjected oligodendrocytes. An additional question to be answered, therefore, is whether some portion of the MP-bound fluorescence that is associated with microtubules contains viral RNA.

REFERENCES AND NOTES

1. A. J. Maule, *Crit. Rev. Plant Sci.* **9**, 457 (1991); W. J. Lucas and R. I. Gilbertson, *Annu. Rev. Phytopathol.* **32**, 387 (1994); B. Epel, *Plant Mol. Biol.* **26**, 1343 (1994).
2. C. M. Deom, M. J. Oliver, R. N. Beachy, *Science* **237**, 389 (1987).
3. M. C. Deom, M. Lapidot, R. N. Beachy, *Cell* **69**, 221 (1992).
4. K. Tomenius, D. Clapham, T. Meshi, *Virology* **160**, 363 (1987); P. Moore, C. A. Frenczik, C. M. Deom, R. N. Beachy, *Protoplasma* **170**, 115 (1992).
5. S. Wolf, C. M. Deom, R. N. Beachy, W. J. Lucas, *Science* **246**, 377 (1989).
6. C. M. Deom *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3284 (1990).
7. H. S. Padgett and R. N. Beachy, *Plant Cell* **5**, 577 (1993).
8. M. Chalfie, Y. Tu, G. Euskirchen, W. W. Ward, D. C. Prasher, *Science* **263**, 802 (1994); T. Stearns, *Curr. Biol.* **5**, 262 (1995).
9. Preparation of protoplasts and electroporation transfer of in vitro–transcribed viral RNA was performed as described [Y. Watanabe, T. Ohno, Y. Okada, *Virology* **120**, 478 (1982); Y. Watanabe, T. Meshi, Y. Okada, *FEBS Lett.* **219**, 65 (1987)]. Infected protoplasts were cultured at 23°C.
10. Constructs pOb-M:Gfus and pObΔC-GFP were described in B. L. Epel, H. S. Padgett, M. Heinlein, R. Beachy, *Gene*, in press. We constructed pTMV-M:Gfus by inserting a polymerase chain reaction–amplified portion of the TMV genome containing the MP gene upstream of the GFP gene as an in-frame fusion. Thirty-one nucleotides from the 3′ terminus of the MP open reading frame encoding the coat protein (CP) gene subgenomic promoter were deleted and replaced by 40 nucleotides of the pGEX-KG cloning vector that encode a flexible linker peptide. The gene fusion was inserted into pU3/12-RO-PL, a derivative of the infectious cDNA clone pU3/12 [C. A. Holt and R. N. Beachy, *Virology* **181**, 109 (1991)] encoding wild-type TMV, from which the MP and CP gene sequences had been deleted and replaced with a polycloning site. The resulting construct, pTMV-M:Gfus, contained a fusion of the MP and GFP open reading frames, the transcription of which remained under the control of the subgenomic promoter that controls production of the MP mRNA. The construct pTMVΔC-GFP was designed to express the GFP from the highly active CP promoter and was constructed by subcloning the GFP gene from pGFP-01 into the Sna BI site of pTMVΔC-SB, a derivative of pU3/12 from which CP gene sequences have been deleted (M. Heinlein, B. L. Epel, H. S. Padgett, R. N. Beachy, unpublished results).
11. Microscopy was performed with a Nikon Optiphot 2UD microscope. For visualization of GFP fluorescence, a FITC filter cube (470- to 490-nm excitation filter, 505-nm dichroic mirror, and 520-nm barrier filter) was used.
12. For immunostaining, protoplasts were harvested at 16 hpi, fixed for 30 min in 50 mM phosphate buffer (pH 6.7) containing 3% paraformaldehyde and 5 mM EGTA, and then spun onto polylysine-coated slides and dried. The protoplasts were then extracted for 10 min with cold methanol. Antibodies were applied in phopshate-buffered saline, pH 7.0, containing 0.5% Tween-20 and 5 mM EGTA, and all washes were performed in the same buffer. The samples were mounted in Mowiol (Calbiochem) containing 2.5% 1,4-diazobicyclo-[2.2.2.]-octane (DABCO) as an antifade reagent.
13. A 510- to 560-nm excitation filter, a 575-nm dichroic mirror, and a 590-nm barrier filter were used to examine rhodamine fluorescence.
14. M. Heinlein, B. L. Epel, H. S. Padgett, R. N. Beachy, data not shown.
15. C. W. Lloyd, A. R. Slabas, A. J. Powell, S. B. Lowe, *Planta* **147**, 500 (1980); R. E. Williamson, *Int. Rev. Cytol.* **129**, 135 (1991).
16. T. Akashi, S. Kawasaki, H. Shibaoka, *Planta* **182**, 363 (1990).
17. Prefixation of F-actin and buffer conditions were as described [J. Katsuta, Y. Hashiguchi, H. Shibaoka, *J. Cell Sci.* **95**, 413 (1990)].
18. P. K. Hepler, *Protoplasma* **111**, 121 (1982); K. J. Oparka, D. A. M. Prior, J. W. Crawford, *Plant Cell Env.* **17**, 163 (1994).
19. T. Akashi and H. Shibaoka, *J. Cell Sci.* **98**, 169 (1991); H. Quader, A. Hoffman, E. Schnepf, *Eur. J. Cell Biol.* **44**, 17 (1987); I. K. Lichtscheidl and W. G. Url, *Protoplasma* **157**, 203 (1990); I. K. Lichtscheidl, S. A. Lancelle, P. K. Hepler, *ibid.* **155**, 116 (1990).
20. P. K. Hepler, B. A. Palevitz, S. A. Lancelle, M. M. McCauley, I. Lichtscheidl, *J. Cell Sci.* **96**, 355 (1990).
21. T. A. Schroer and M. P. Sheetz, *Annu. Rev. Physiol.* **53**, 629 (1990); J. Lippincott-Schwartz, N. B. Cole, A. Marotta, P. A. Conrad, G. S. Bloom, *J. Cell. Biol.* **128**, 293 (1995).
22. K. Kristensson, E. Lycke, M. Ryotta, B. Svennerholm, A. Vahlne, *J. Gen. Virol.* **67**, 2023 (1986); S. Dales and Y. Chardonnet, *Virology* **56**, 465 (1973); A. P. de Matos and Z. G. Carvalho, *Biol. Cell* **78**, 229 (1993); U. I. Heine, A. E. Demsey, R. W. Tucker, A. F. Bykovsky, *J. Gen. Virol.* **66**, 275 (1985); E. Lycke and H. Tsiang, *J. Virol.* **61**, 273 (1987); K. S. Topp, L. B. Meade, J. H. LaVail, *J. Neurosci.* **14**, 318 (1994); B. Norrild, V. P. Lehto, I. Virtanen, *J. Gen Virol.* **67**, 97 (1986); M. J. Rindler, I. E. Ivanov, D. D. Sabatini, *J. Cell Biol.* **104**, 231 (1987).
23. A. V. Karasev, A. S. Kashina, V. I. Gelfand, V. V. Dolja, *FEBS Lett.* **304**, 12 (1992).
24. M. Terasaki, L. B. Chen, K. Fujiwara, *J. Cell Biol.* **103**, 1557 (1986); S. L. Dabora and M. P. Scheetz, *Cell* **54**, 27 (1988); R. D. Vale and H. Hotani, *J. Cell Biol.* **107**, 2233 (1988).
25. S. A. Lancelle, M. Cresti, P. K. Hepler, *Protoplasma* **140**, 141 (1987).
26. W. Knebel, H. Quader, E. Schnepf, *Eur. J. Cell Biol.* **52**, 328 (1990).
27. V. Citovsky *et al.*, *Cell* **60**, 637 (1990); V. Citovsky *et al.*, *Plant Cell* **4**, 397 (1992).
28. W. E. Theurkauf, B. M. Alberts, Y. N. Jan, T. A. Jongens, *Development* **118**, 1169 (1993); J. E. Wilhelm and R. D. Vale, *J. Cell Biol.* **123**, 269 (1993).
29. K. Ainger *et al.*, *J. Cell Biol.* **123**, 431 (1993).

30. We thank J. Harper for providing pGFP-01, which contains the GFP coding sequence obtained from R. Tsien, and G. Klier for assistance with confocal microscopy. Supported by NSF grants MCB 9209530 and MCB 9317368 and the Scripps Family Chair to R.N.B. M.H. was supported by the Deutsche Forschungsgemeinschaft and the Human Frontier Science Program Organization.

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