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Inhibition of Host Gene Expression Associated with Plant Virus Replication

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Pea seed–borne mosaic virus (PSbMV) RNA replication in pea cotyledonary tissues was restricted largely to a zone of cells close to the infection front. In situ hybridization probes representing nine genes from two pathways of metabolism failed to detect RNA transcripts within this zone, although transcripts were found in similar amounts in tissues on either side of the zone. Thus, in common with some animal viruses, PSbMV transiently suppresses the expression of host genes. Host protein accumulation was also affected. These observations provide insights into virus-plant interactions and symptom expression.

Viruses exert their negative effects by influencing the metabolism of the host (*1*, *2*), but the mechanism is poorly understood for plant viruses. Although alterations in plant gene expression have been linked to viral infection, they have not been related to specific events in virus multiplication (*3*). We used here a histochemical approach to identify cells in which virus is actively replicating and to study the associated activity of host genes.

Pea seed–borne mosaic virus (PSbMV) is a member of the potyviridae (*4*) and has a positive-sense RNA genome of approximately 10 kilobases (kb). It is transmitted in seeds after infection of the embryo early in development (*5*). As the infection proceeds, the virus invades the cotyledon on an advancing front (*6*).

To identify cells supporting active virus replication, we probed sections of immature infected pea embryos for virus coat protein with a PSbMV antibody, and for the positive and negative senses of the RNA genome with the use of in situ hybridization (*7*). Detection of both coat protein and positive PSbMV RNA showed that the virus had accumulated in tissues proximal to the contact point with the embryonic axis, leaving a distal portion of cotyledon uninfected (Fig. 1, B and C). In the infected area, there was a uniform accumulation of virus with a sharp differentiation between infected and uninfected areas, which suggests that PSbMV replication is rapid. Estimates for the rate of advancement of infections by a related potyvirus, tobacco etch virus, suggest that the virus infects approximately one new cell every 2 hours (*8*).

Negative sense RNA is part of the replicative form of the virus (*9*). Negative sense RNA is mostly single-stranded in tissues involved in active viral RNA replication but is predominantly part of a duplex when RNA replication is completed (*10*). In situ hybridization to detect PSbMV negative sense RNA identified a zone of tissue along the periphery of the infected area where this RNA was most abundant, with much smaller amounts detected within the infected area (Fig. 1D). Hence, cells in this peripheral zone were those most recently involved in active viral RNA replication. We could not distinguish whether the weak signal seen farthest from the infection front was due to degradation of the replicative form of viral RNA or to our inability to denature fully double-stranded RNA.

To investigate the consequence of virus replication on the metabolism of the host, we used the same approach to analyze the expression of nine pea genes in tissues of the cotyledon. Five genes encoded the pea seed storage proteins legumin (three members of one gene family) (*11*), vicilin (*12*), and convicilin (*13*). Four genes encoded isoforms of granule-bound starch synthase (GBSSI and GBSSII) (*14*) and the large and small subunits of adenosine diphosphate glucose pyrophosphorylase (ADPGP) (*15*), all of which are enzymes involved in starch biosynthesis. These genes represented two pathways of metabolism, different steady-state amounts of transcripts, and different timing of expression in relation to embryo development (*14*). Sections derived from uninfected and PSbMV-infected embryos were treated with strand-specific probes for PSbMV RNA or for transcripts of the nine host genes (*16*). In sections of healthy tissue, host transcripts showed uniform distribution in the cortical tissue of the cotyledon, with greater expression of the seed storage protein genes in the peripheral and surface layers of the cotyledon (Fig. 2). In contrast, in sections cut from infected embryos, a band of cotyledonary cells with barely detectable host gene transcripts was consistently seen. This band was seen regardless of whether the transcripts were normally found in large (for example, convicilin) (6) or small (for example, ADPGP) (6) amounts. In the cells that had been infected earlier and in which the amount of PSbMV negative-sense RNA was declining, transcripts of the nine host genes accumulated to an extent comparable to that found in uninfected cells of the same cotyledon section (Fig. 2, D through L). From the relative positions of this transcript-deficient band, the PSbMV-infected area, and the periph-

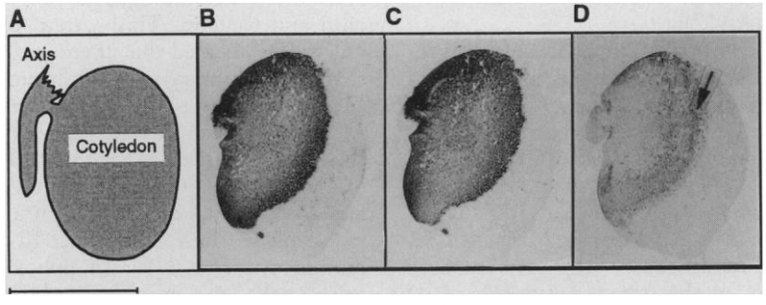

Fig. 1. Localization of PSbMV RNA and capsid protein in infected pea cotyledons. A PSbMV-infected pea embryo was sectioned close to the union between the embryonic axis and the cotyledon as shown in (**A**), and serial sections were probed for the location of virus-specific protein and RNA. Virus capsid protein (**B**) was detected by immunohistochemistry with a monoclonal antibody, whereas virus positive- (**C**) and negative- (**D**) sense RNA were detected by in situ hybridization (*7*). There was a correlation between the distribution of viral capsid protein and the genomic positive-sense RNA and a sharp boundary between the areas of infected and uninfected tissue. The faint spots of signal seen in the uninfected area result from loosened cells becoming displaced to distant areas during sectioning and processing. Viral negative-sense RNA accumulation was greatest close to the infection boundary, indicating a narrow zone of active viral RNA replication (arrow). Size bar: 5 mm.

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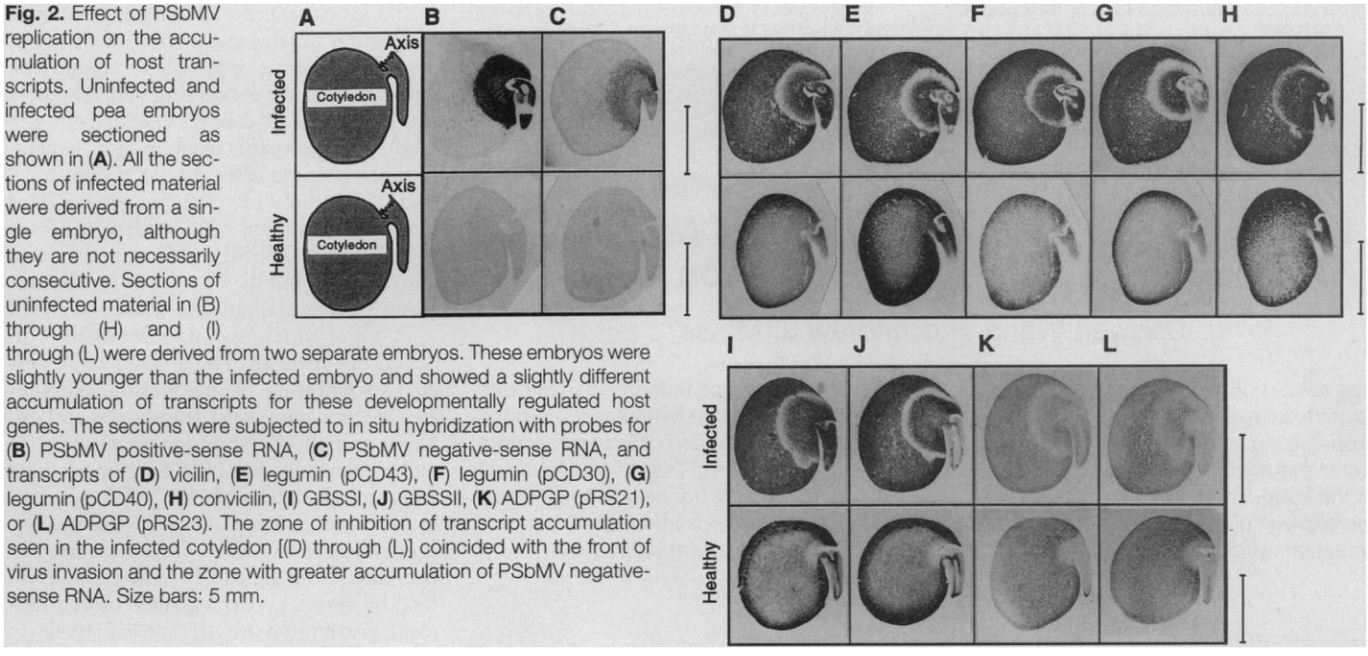

Fig. 2. Effect of PSbMV replication on the accumulation of host transcripts. Uninfected and infected pea embryos were sectioned as shown in (**A**). All the sections of infected material were derived from a single embryo, although they are not necessarily consecutive. Sections of uninfected material in (B) through (H) and (I) through (L) were derived from two separate embryos. These embryos were slightly younger than the infected embryo and showed a slightly different accumulation of transcripts for these developmentally regulated host genes. The sections were subjected to in situ hybridization with probes for (**B**) PSbMV positive-sense RNA, (**C**) PSbMV negative-sense RNA, and transcripts of (**D**) vicilin, (**E**) legumin (pCD43), (**F**) legumin (pCD30), (**G**) legumin (pCD40), (**H**) convicilin, (**I**) GBSSI, (**J**) GBSSII, (**K**) ADPGP (pRS21), or (**L**) ADPGP (pRS23). The zone of inhibition of transcript accumulation seen in the infected cotyledon [(D) through (L)] coincided with the front of virus invasion and the zone with greater accumulation of PSbMV negative-sense RNA. Size bars: 5 mm.

eral zone involved in active PSbMV replication, it appeared that the cells deficient in host transcripts were those most active in viral RNA replication. The transcript-deficient band encompassed between four and eight cell layers (*17*).

To confirm the link between reduced transcript accumulation and the presence of the replicative form of the viral RNA, we mixed the hybridization probes for host and viral RNAs (Fig. 3). When consecutive sections of infected cotyledonary tissue were analyzed with mixtures of probes for positive-sense PSbMV RNA and legumin (Fig. 3E) or negative-sense PSbMV RNA and legumin (Fig. 3F), the transcript-deficient band, apparent with the legumin probe alone (Fig. 3D), disappeared. Identical results were obtained in mixed probings for PSbMV RNA and GBSSI RNA (*17*).

We investigated the extent of host protein accumulation by subjecting consecutive sections to immunohistochemical analysis with antibodies specific for vicilin, legumin, GBSSI and GBSSII, and ADPGP (*18*). In uninfected embryos, the host proteins were uniformly distributed through the cortical tissue of the cotyledon and for all except ADPGP were more abundant in the surface layers (Fig. 4, D through H). In contrast, PSbMV-infected embryos showed a different pattern of protein accumulation. For all the proteins [except ADPGP (Fig. 4H)], there was a variable decrease in accumulated protein at the front of, but within, the zone of viral RNA replication (Fig. 4, D through G). Inside the infected area, closer to the embryonic axis, this was followed by a more intense staining, particularly for vicilin and legumin. This pattern of accumulation indicated that there was a more than compensatory increase in protein in cells just behind the infection front.

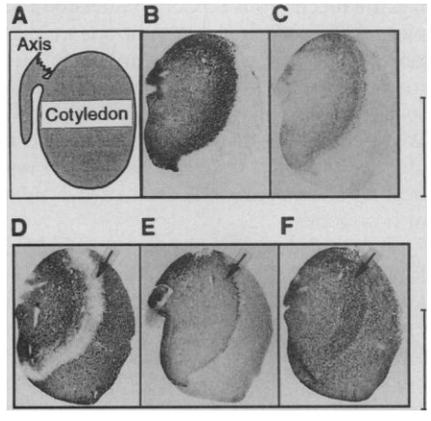

Fig. 3. Confirmation of the coincidence between the host transcript-deficient zone and the location of PSbMV replication. A PSbMV-infected pea embryo was sectioned as shown in (**A**), and serial sections were probed with virus-specific probes for positive-sense RNA (**B**) or negative-sense RNA (**C**), a legumin (pCD43) RNA probe (**D**), or a combination of virus and host gene probes (**E** and **F**), with the use of in situ hybridization. In (E), the combination of probes for legumin RNA and for PSbMV positive-sense RNA obliterates the clear zone seen in (D) (arrow). The signal from PSbMV positive-sense RNA is much stronger than that for legumin RNA; to see the relative signals most clearly, we developed the color reaction in (E) for a shorter time than in (B) or (D). In (F), the combination of the probe for legumin RNA with the probe for PSbMV negative-sense RNA precisely filled the clear zone seen in (D) (arrow). Size bars: 5 mm.

What mechanisms are responsible for the observed effects of PSbMV replication on host gene expression? Picornavirus infection of animal cells results in a drastic reduction in the rates of host RNA and protein synthesis (*19*). In poliovirus-infected cells, the decrease in host RNA synthesis is correlated with degradation of transcription factors by the viral serine protease 3C (*20*), and the decrease in translation is effected by cleavage of a cellular translational component, p220, by the viral serine protease 2A (*21*, *22*). The family potyviridae belongs to the picornavirus superfamily (*23*), and like polioviruses, potyviruses encode two serine-like proteases, P1 and NIa (*24*). Structurally and functionally, the NIa proteinase may resemble the picornaviral 3C proteinase (*24*). Although this implies that PSbMV-encoded products reduce host transcription, we cannot yet rule out that the effect is rather to increase degradation of host mRNAs or to block nuclear export of mature transcripts. That the diverse range of host transcripts, which probably vary in their stability in uninfected tissues, all show an abrupt disappearance with the onset of viral RNA replication suggests that active messenger RNA degradation may be important. If there is a direct effect from a viral gene product or products, then we must explain the almost equally rapid restoration of transcript levels after viral RNA replication has declined, although many of the potyviral gene products persist for long periods in vivo (*4*).

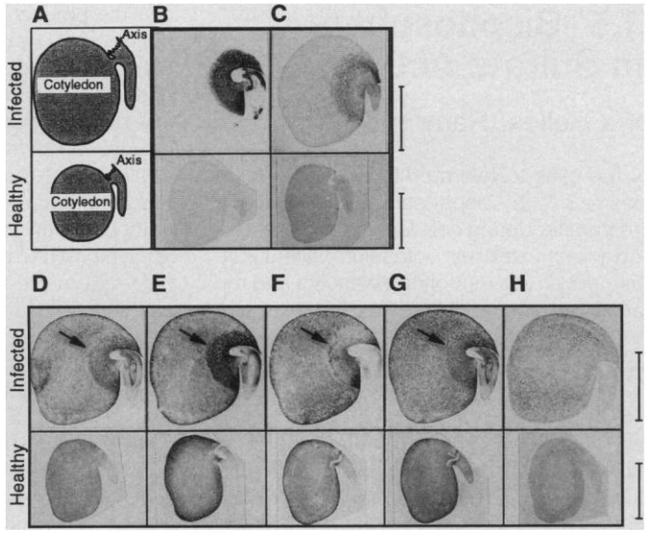

Fig. 4. Effect of PSbMV replication on host protein accumulation. Uninfected and infected pea embryos were sectioned as shown in (**A**). All sections of infected or uninfected material were derived from single embryos. Sections were probed for PSbMV positive-sense RNA (**B**) or PSbMV negative-sense RNA (**C**) with the use of in situ hybridization or with antibodies to pea gene products (D through H) with the use of immunohistochemistry. The antibodies were for (**D**) vicilin, (**E**) legumin (this antibody did not distinguish between the products of the three legumin genes), (**F**) GBSSI, (**G**) GBSSII, or (**H**) ADPGP (this antibody detects both subunits of the enzyme) (*26*). For all the gene products, except ADPGP, there was a slight decrease in accumulated protein (arrows) in the region of the zone of active virus replication, which was followed temporally by a greater accumulation in the remainder of the infected area. Size bars: 5 mm.

The effect of PSbMV replication on the accumulation of host proteins is more complex. It is likely that these proteins continue to accumulate in uninfected cells while translation in cells at the invasion front has ceased because of an absence of transcript. The temporal delay in reestablishing host gene expression results in a relatively smaller accumulation of host proteins in these cells. The absence of an effect on ADPGP protein indicates that this phenomenon is not seen for all host proteins and may reflect their relative rates of synthesis as the wave of infection passes. The elevated accumulation of vicilin, legumin, and GBSSI and GBSSII peptides (but not of their transcripts) in cells later in the infection process suggests an imperfect mechanism of translational control in the recovery of infected cells from the inhibitory effects of virus replication.

The consequences of these profound disturbances on the balance of normal cellular processes are unknown, but it is conceivable that they could trigger events that result in the expression of virus-induced symptoms. Physiological changes that occur after plant virus infection may follow a series of programmed changes initiated soon after virus invasion of an uninfected cell (*25*). Such changes could be consistent with the increase in protein accumulation observed here for PSbMV in peas.

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7. Pea plants (*Pisum sativum* cv. Vedette) were infected with PSbMV (isolate 28) (*5*), and embryos between 150 and 210 mg were harvested for analysis; fresh mature embryos of this variety weigh approximately 250 mg. Immunohistochemistry with a monoclonal antibody specific for PSbMV coat protein and in situ hybridization with a negative-sense RNA probe specific for PSbMV positive-sense RNA were carried out as described (*5*) on sections (15 μm) of cotyledonary tissue. For detecting negative-sense PSbMV RNA, the in situ hybridization protocol in (*5*) was modified by heat denaturation immediately after application of the hybridization mix to the sections. The denaturation (100°C for 15 min) was carried out in a sealed metal box that contained filter papers soaked in 2× SSC (0.03 M sodium citrate and 0.3 M sodium chloride) and 75% formamide. The box was then transferred to 37°C for 30 min, then to 50°C. For maximum sensitivity, RNA transcript probes from two plasmids, R3 and R7, were combined and used at a concentration of 500 ng/ml of hybridization mix per kilobase of target sequence. These plasmids contained PSbMV sequences [E. Johansen, O. F. Ramussen, M. Heide, B. Borkhardt, *J. Gen. Virol.* **72**, 2625 (1991)] from nucleotides 1 to 3567 and 3440 to 9928, respectively, behind the phage T7 RNA polymerase promoter.
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14. Plasmids pPEA and pPEA7 contained, respectively, 2.04 kb of GBSSI cDNA and 3.0 kb of GBSSII cDNA cloned into pBluescript(−) [I. Dry *et al.*, *Plant J.* **2**, 193 (1992)].
15. Plasmids pRS21 and pRS23 contained, respectively, 2.0 kb of ADPGP large subunit cDNAs and 1.4 kb of ADPGP small subunit cDNAs cloned in pBluescript(−) (R. Burton and C. Martin, unpublished material).
16. Host RNAs were detected with RNA probes prepared from the nine cDNAs and with use of the protocol described in (*5*). We obtained infected embryos and control, uninfected embryos from the same infected plants. This was possible because PSbMV shows only approximately 60% seed transmission and embryo infection (*5*). Hybridization with positive-sense RNA probes did not produce a detectable signal (*17*).
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18. Immunohistochemistry with rabbit polyclonal antibodies specific for vicilin, legumin, GBSSI and GBSSII, and ADPGP was carried out on sections of pea cotyledons with a protocol modified from the one described in (*5*). The immunoglobulin G fraction (1 mg/ml) of antibodies to vicilin, legumin, and ADPGP was used at a concentration of 2 μg/ml. Unfractionated antisera to GBSSI and GBSSII were used in a 1:500 dilution. To detect the antigen-antibody complex, we used protein A–alkaline phosphatase conjugate (Sigma) at a concentration of 0.2 μg/ml. Control, uninfected embryos (*16*) were processed in parallel. Rabbit preimmune antiserum used under identical conditions produced no significant signals (*17*).
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