Baculovirus Expression and Processing of Tomato Spotted Wilt Tospovirus Glycoproteins

Scott Adkins, Tae-Jin Choi, Barbara A. Israel, Murali D. Bandla, Kathryn E. Richmond, Kevin T. Schultz, John L. Sherwood, and Thomas L. German

First, second, fifth, and eighth authors: Department of Plant Pathology, University of Wisconsin-Madison; third and sixth authors: Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison 53706; fourth and seventh authors: Department of Plant Pathology, Oklahoma State University, Stillwater 74078.

Current address of T.-J. Choi: National Fisheries University of Pusan, Department of Microbiology, 599-1, Daeyeon 3-Dong, Nam-Gu, Pusan 608-737, Korea. Current address of K. T. Schultz: Merck Research Laboratories, P.O. Box 2000 RY33-216, Rahway, NJ 07065.

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ABSTRACT

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The open reading frame encoding the polyprotein precursor to the tomato spotted wilt tospovirus (TSWV) G1 and G2 glycoproteins was cloned as cDNA and used to produce a recombinant baculovirus. Protein expression was monitored in recombinant baculovirus-infected *Spodoptera frugiperda* IPLB-SF21 (SF21) cells with monoclonal antibodies raised to TSWV G1 and G2. Western blot analysis demonstrated expression, proteolytic processing, and glycosylation of the G1G2 polyprotein to yield proteins with serological specificities and electrophoretic mobilities similar to the G1 and G2 proteins found in TSWV virions. Indirect immunofluorescence localization of G1 and G2 in infected SF21 cells revealed accumulation of the glycoproteins in the plasmalemma. These experiments establish that the glycoproteins of TSWV, a plant-infecting virus in the family Bunyaviridae, can be processed in the absence of other viral proteins.

Tomato spotted wilt tospovirus (TSWV) is the type member of the genus *Tospovirus* in the family Bunyaviridae. Viruses in this family have tripartite genomes of negative and ambisense RNA and generally are arthropod-borne (8). Although most viruses in the Bunyaviridae infect vertebrates (41), TSWV infects more than 650 species of plants (12) as well as its thrips (Thysanoptera: Thripidae) vector (50,56). TSWV virions are enveloped by a membrane of host origin that contains two viral-encoded glycoproteins (G1 and G2) and encloses a putative RNA-dependent RNA polymerase protein (L) and three genomic RNAs (small [S], medium [M], and large [L]), each individually encapsidated by many copies of the nucleocapsid (N) protein (11). The ambisense 2.9-kb S RNA encodes a 52.4-kDa nonstructural (NSs) protein in the viral (v) sense and the 29-kDa N protein in the viral complementary (vc) sense (6). The 4.8-kb M RNA also is ambisense and encodes a 33.6-kDa nonstructural (NSm) protein in the v sense and a 127.4-kDa polyprotein precursor to G1 and G2 in the vc sense (20). The negative-sense 8.9-kb L RNA contains a single open reading frame (ORF) in the vc sense that encodes the 331.5-kDa L protein (5).

The vertebrate-infecting viruses in the Bunyaviridae encode two glycosylated membrane proteins analogous to TSWV G1 and G2, although there are several distinct organizations in the ORFs encoding these proteins (9). Heterologous expression (22,27,28,33, 39) and antibody-based approaches (24,25) suggest that the viral glycoproteins function in two phases of the infection cycle: maturation/assembly of virions and attachment to cell surface receptors (31,34).

Although there is a plethora of experimental data regarding the roles of G1 and G2 in the vertebrate-infecting bunyaviruses, little progress has been made in determining the function(s) of these proteins for viruses in the genus *Tospovirus*. The maturation site for the tospoviruses has not been determined, and the assembly process of TSWV has never been observed in plant (18) or thrips (51) cells despite more than 30 years of electron microscopic observation (17). Tospovirus glycoproteins had been observed only on membranes of intact virions in plant or thrips cells until recently, when TSWV glycoproteins were immunolocalized to membranes thought to be part of the Golgi complex in thrips cells (51). TSWV virions have been observed in membrane-bound compartments of the endoplasmic reticulum (ER) in plant cells, suggesting the ER as a potential maturation/assembly site (10,18). These two disparate observations comprise the current knowledge of tospovirus maturation.

Specific roles for glycoproteins in TSWV replication, pathogenesis, or vector relations have not been clearly established. Repeated mechanical inoculation of TSWV on plants led to envelope-deficient mutants that lost the M RNA sequences encoding the glycoproteins (35). Although these mutants infected plants in a manner indistinguishable from wild-type TSWV, they were no longer thrips-transmissible (55), suggesting that glycoproteins are required for the thrips' portion of the TSWV disease cycle, possibly for acquisition of the virus. The absence of intact TSWV virions between the gut and salivary gland and the sequestration of most glycoproteins in vacuoles (except for minor amounts ob-

Corresponding author: T. L. German; E-mail address: tlg@plantpath.wisc.edu

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served in putative Golgi membranes) in infected thrips cells (51) suggests that glycoproteins function in viral entry and exit.

We report the cloning of the ORF encoding TSWV glycoproteins as cDNA, production of monoclonal antibodies (MAbs) specific for TSWV G1 or G2, generation of a recombinant baculovirus containing the cloned G1G2 ORF, and analysis of expression and localization of these proteins in insect cells. The data and insights these methods offer into TSWV glycoprotein processing and maturation relative to the vertebrate-infecting viruses of the family Bunyaviridae are discussed. Preliminary reports on the production of MAbs to TSWV G1 or G2 (2) and expression of G1 and G2 in the baculovirus system (4) have been made.

MATERIALS AND METHODS

Virus isolate and plant material. A TSWV isolate (TSWV-MT2) collected on the Hawaiian island of Maui was used for all experiments. Maintenance and propagation of the virus in Jimsonweed (*Datura stramonium* L.) plants was as previously described (49,50). Leaves were harvested just prior to maximal symptom expression, and TSWV virions were isolated by the procedures of Gonsalves and Trujillo (13).

Production of MAbs to glycoproteins. Glycoproteins were isolated from TSWV virions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 9% minigels prepared per the manufacturer's instructions for the ChromaPhor system (Promega, Madison, WI). One hundred to one hundred fifty micrograms of total virion protein was loaded per minigel. Immediately after electrophoresis, visualized G1 and G2 were excised, and the proteins were recovered in 1× phosphate buffered saline (PBS = 0.14 M NaCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.5 mM KCl, pH 7.5) instead of ammonium bicarbonate buffer (pH 7.8), as recommended by the manufacturer. Eluted proteins were concentrated using spin concentrators with 10-kDa cutoffs and stored at –20°C.

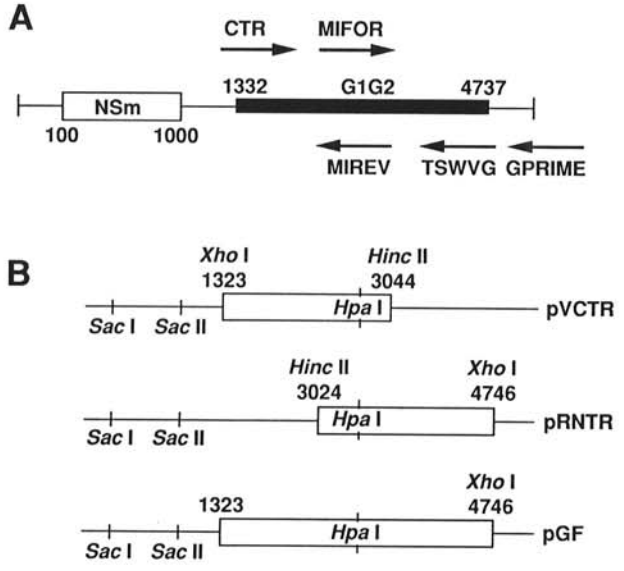

Fig. 1. Cloning of the tomato spotted wilt virus (TSWV) G1G2 open reading frame (ORF). **A,** The 4,821-nt viral (v) sense TSWV medium (m) RNA with the positions of the positive-sense NSm ORF and negative-sense G1G2 ORF indicated. Primers for cloning of the G1G2 ORF (described in text) are denoted by arrows above (v sense) or below (viral complementary [vc] sense) the RNA. **B,** The sequence encoding the C-terminal half of the G1G2 polyprotein (nt 1323–3044) was cloned as pVCTR from viral RNA by reverse transcription-polymerase chain reaction (RT-PCR) with primers MIREV and CTR. The sequence encoding the N-terminal half of the G1G2 polyprotein (nt 3024–4746) was cloned as pRNTR from viral RNA by RT-PCR with primers TSWVG and MIFOR after an unsuccessful attempt with primers GPRIME and MIFOR. The unique *Hpa*I site (indicated with a tick mark) in the overlapping region of pRNTR and pVCTR was used to join them and produce pGF, a complete G1G2 ORF clone. The *Xho*I/*Sac*II fragment of pGF was used for construction of the baculovirus transfer vector described in text. All coordinates are numbered according to nucleotide position in v sense RNA (20).

Cloning of the TSWV G1G2 ORF. The G1G2 ORF was cloned as two overlapping cDNAs with primers oriented as shown in Figure 1A and based on the sequence of the M RNA of the Brazilian TSWV isolate BR-01 (20). RNA was extracted from TSWV virions as previously described (50). GPRIME or MIREV primer (50 pmol) was used for first-strand cDNA synthesis by reverse transcription of TSWV RNA as previously described (50). Primer GPRIME (5′-CGCGGATCCAGAGCAATCAGTGCAAACAA-3′) is complementary to the 3′ end of the M RNA (nucleotides [nt] 4802 to 4821) and contains a *Bam*HI site (underlined) and three extra nucleotides to facilitate cloning. Primer MIREV (5′-CTTTAGGGTTAACATCAGTTG-3′) is complementary to M RNA nt 3024 to 3044 and contains an *Hpa*I site (underlined).

After preheating cDNA at 95°C for 5 min, polymerase chain reaction (PCR) was used for amplification, with denaturation at 90°C for 30 s, annealing at 55°C for 45 s, and extension at 75°C for 1 min. A 3-min extension at 72°C followed 40 cycles of PCR. Primers GPRIME and MIFOR (complementary to MIREV) were used for amplification of the sequence encoding the N-terminal half of the G1G2 polyprotein, whereas primers MIREV and CTR (5′-CTGTctcgAGCATCTTCAGACAAGGTG-3′) were used for amplification of the sequence encoding the C-terminal half of the polyprotein. Primer CTR is located at nt 1315 to 1341 of the viral-sense M RNA but contains several mismatched nucleotides (lowercase) to include an *Xho*I site (underlined).

Products from MIREV/CTR PCR reactions were digested with *Xho*I and ligated into *Xho*I/*Hinc*II-digested pBluescript KS(+) (pBS) (Stratagene, La Jolla, CA). The recombinant plasmid was designated pVCTR (Fig. 1B) and was used to transform *Escherichia coli* strain DH5α. Products from GPRIME/MIFOR PCR reactions were digested with *Bam*HI and *Hinc*II and ligated into *Bam*HI/*Hinc*II-digested pBS. No colonies were recovered when *E. coli* was transformed with this recombinant plasmid. However, the entire GPRIME/MIFOR PCR product was successfully cloned as three separate pieces by digestion with *Bam*HI and *Spe*I, *Spe*I and *Hin*dIII, or *Hin*dIII and *Hinc*II prior to generation of recombinant plasmids. This suggested the presence of interacting toxic sequence elements in this region of the M RNA.

Primer TSWVG (5′-GGGTActCgagaAAtATGAGAATTCTAAAAC-3′), therefore, was designed to exclude most of the 3′ untranslated region of the M RNA and was used with primer MIFOR

to clone the N-terminal half of the G1G2 polyprotein, as described above. Primer TSWVG is complementary to M RNA nt 4722 to 4752 but has several mismatched nucleotides (lowercase) to include an *Xho*I site (underlined) and the translation-enhancing sequence for the *Autographa californica* nuclear polyhedrosis virus (Ac*M*NPV) polyhedrin gene (bold). TSWVG/MIFOR PCR products were processed in a manner identical to MIREV/CTR PCR products, and the recombinant plasmid was designated pRNTR (Fig. 1B).

The TSWV sequence was excised from pVCTR, blunted with mung bean nuclease, and ligated into *Eco*RV-digested pBS to facilitate assembly of a full-length G1G2 ORF clone. This TSWV sequence was excised at the unique *Hpa*I site in the G1G2 ORF and a unique *Sac*I site in the pBS polylinker and ligated into *Hpa*I/*Sac*I-digested pRNTR to generate pGF, a clone of the complete G1G2 ORF (Fig. 1B). Correct assembly of the fragments was verified by dideoxy sequencing (40) and comparison to the published M RNA sequence, described above, by programs provided by the Genetics Computer Group (Madison, WI).

The N terminus of the G1G2 polyprotein and the 3′ untranslated region of the M RNA (5′ untranslated region of the G1G2 mRNA) were analyzed for sequence elements conferring the apparent toxicity observed during cloning. A 30-amino acid hydrophobic region at the N terminus of the G1G2 polyprotein is the putative signal sequence for cotranslational insertion of the glycoproteins in the ER (20). Toxicity or lethality observed upon overexpression of other viral glycoproteins in *E. coli* has been associated with regions encoding analogous hydrophobic signal sequences (38,44). Therefore, this putative signal sequence was subcloned from pRNTR into the pET11a inducible expression vector (Novagen, Madison, WI) and tested for toxicity to *E. coli* strain DH5α. Bacterial growth was measured by optical density after transformation with the recombinant plasmid and induction of protein expression as previously described (50). Sequence analysis revealed the sequences TTGAGA at –41 and TGTAAT at –19 in the 5′ untranslated region of the G1G2 mRNA, which are similar to the –35 and –10 prokaryotic promoter consensus sequences of TTGACA and TATAAT, respectively. This region was subcloned from the *Bam*HI-*Spe*I fragment of the GPRIME/MIFOR PCR product into pBS upstream of the β-glucuronidase (GUS) reporter gene. Its ability to function as a prokaryotic promoter was assayed by transforming *E. coli* with the recombinant plasmid and measuring GUS activity.

Preparation of recombinant baculovirus. An *Xho*I/*Sac*II fragment of pGF was inserted into the *Xho*I/*Sac*II-digested baculovirus transfer vector, pEV/35K (23), to generate pEVG. The lepidopteran cell line *Spodoptera frugiperda* IPLB-SF21 (SF21) (53) was propagated at 27°C in TC100 growth medium (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS). SF21 cell cultures were transfected with 20 μg of *Nde*I-linearized pEVG transfer vector and 1 μg of *Bsu*36I-digested Ac*M*NPV mutant vΔ35K/lacZ DNA (14) with Lipofectin (Life Technologies) as previously described (7). A recombinant virus was plaque purified (23), amplified (32), and designated TSWVGP.

Expression of the TSWV G1G2 ORF from TSWVGP. Monolayers of SF21 (3×10^6) cells were inoculated with TSWVGP at a multiplicity of infection (MOI) of 10 according to standard protocols (32). For some treatments, growth medium contained tunicamycin (an inhibitor of N-linked glycosylation) at 25 μg/ml. SF21 cells were incubated at 27°C and harvested at 36, 48, and 72 h after inoculation. Mock- or wild-type Ac*M*NPV-infected SF21 cells served as negative controls and were harvested at 72 h. SF21 cells were dislodged from tissue-culture dishes, pelleted, washed with PBS, suspended in 1% SDS (wt/vol)/2.5% β-mercaptoethanol (vol/vol), and heated at 100°C for 5 min. Cell lysates were diluted with 4× Laemmli buffer (21), and 7×10^4 cell equivalents were analyzed by electrophoresis on 7.5% SDS-PAGE gels.

After electrophoresis, proteins were transferred to Hybond C-Super nitrocellulose membranes (Amersham, Arlington Heights, IL) as described previously (50). Pooled hybridoma supernatants containing MAbs against either TSWV G1 or G2 were used for detection of antigens, as described by Ullman et al. (50), with 5% nonfat dried milk (wt/vol) and ECL chemiluminescent detection (Amersham) in place of gelatin and colorimetric detection.

Indirect immunofluorescence assays (IFA). SF21 cells were grown to monolayers on #1 glass coverslips in individual wells of six-well plastic tissue-culture dishes as described above. The cells were inoculated with TSWVGP or wild-type Ac*M*NPV at MOI > 1. The cells were incubated at 27°C for 24 to 72 h and fixed for 10 min with either a 1:1 mixture of methanol/acetone or 2% paraformaldehyde (wt/vol). After fixation, coverslips were incubated for 1 h with 20% normal goat serum (vol/vol) (Sigma Chemical Co., St. Louis) in PBS. Pooled hybridoma supernatants containing MAbs against TSWV G1 or G2 (as described above) or anti-feline immunoglobulin (Ig) E MAbs (negative control) were diluted 1:20 in PBS and incubated with the cells for 1 h. The coverslips were washed three times with 20% normal goat serum in PBS and incubated for 1 h with a 1:200 dilution of goat anti-mouse IgG conjugated to fluorescein isothiocyanate (Sigma). The coverslips were washed five times (as described above), mounted on glass slides with glycerol/PBS, and visualized with ultraviolet radiation. All incubations were at room temperature.

RESULTS

Cloning of the TSWV G1G2 ORF. Several perplexing incidents occurred during cloning of the full-length TSWV G1G2 ORF and are worth noting, especially for researchers cloning other viral glycoproteins. Expression of the putative N-terminal signal sequence of the G1G2 polyprotein from pET11a reduced bacterial growth, as measured by optical density, compared to a similar vector construct containing the TSWV NSm ORF (data not shown). An increase in GUS activity was detected when the 5′ untranslated region of the G1G2 mRNA was cloned upstream of the GUS reporter gene, suggesting that this region of the M RNA contains sequence elements capable of directing prokaryotic expression (data not shown). This combination of apparent toxic signal and prokaryotic promoter sequences slowed construction of a full-length G1G2 ORF clone considerably.

Baculovirus expression of G1 and G2. The TSWV G1G2 ORF was assembled as a contiguous cDNA fragment from two overlapping clones (Fig. 1) and was used to generate the recombinant baculovirus TSWVGP. The G1G2 polyprotein was expressed and processed during infection of SF21 cells by TSWVGP as determined by Western blot analysis. A protein migrating at approximately 95 kDa reacted specifically with the anti-G1 MAb pool, and a second protein migrating at approximately 58 kDa reacted specifically with the anti-G2 MAb pool (Fig. 2A and B, lane 3). A third protein of approximately 155 kDa reacted with both MAb pools (Fig. 2A and B, lane 3). Proteins of similar electrophoretic mobility were observed in TSWV virion preparations (Fig. 2A and B, lane 5). The electrophoretic mobility of all three proteins increased with the addition of tunicamycin to the SF21 cell cultures (Fig. 2A and B, lane 4), indicating that all three were glycosylated. No immunoreactive proteins were observed in mock- or wild-type Ac*M*NPV-infected SF21 cells (Fig. 2A and B, lanes 1 and 2). The amount of immunoreactive proteins increased through 72 h postinfection (data not shown).

Localization of G1 and G2 in TSWVGP-infected SF21 cells. Expression of the G1G2 ORF was confirmed by indirect IFA with an anti-G1 MAb pool (Fig. 3). Internal labeling of permeabilized (methanol/acetone fixed) TSWVGP-infected SF21 cells was observed at 24 h postinfection (Fig. 3A). By 48 h postinfection, internal labeling increased in intensity, and labeling of the plasmalemma was apparent (Fig. 3B). Label was concentrated at the plasmalemma by 72 h postinfection (Fig. 3C). Support for this pattern of labeling was provided by observation of nonpermeabi-

lized (paraformaldehyde fixed) TSWVGP-infected SF21 cells (Fig. 3E through H). No labeling was detected until 48 h postinfection, when the plasmalemma began to fluoresce (Fig. 3F). Labeling of the plasmalemma by 60 h postinfection (Fig. 3G) was essentially identical to that observed with the permeabilized SF21 cells. No labeling was observed in wild-type Ac*M*NPV-infected SF21 cells fixed with methanol/acetone or paraformaldehyde (Fig. 3D and H) or when anti-feline IgE MAbs were used as the primary antibody (data not shown). Labeling patterns with the anti-G2 MAb pool were indistinguishable from those observed with the anti-G1 MAb pool.

DISCUSSION

The antiglycoprotein MAbs produced in this study were specific for either G1 or G2 and detected both proteins in TSWV virion preparations (Fig. 2A and B, lane 5). Although G2 migrated in SDS-PAGE gels with an estimated molecular mass of 58 kDa, as previously reported (29,48), G1 was observed at approximately 95 kDa. Although this represents an approximately 20-kDa discrepancy from the often cited molecular mass of 78 kDa for G1 (29,48), the molecular mass of G1 reported in the literature actually varies from 78 to 90 kDa (29,30,48). The difference between the molecular weight of G1 in this study and previous reports, as well as the general inconsistencies in the literature, are likely due to the difficulties inherent in molecular weight estimation of glycoproteins by gel electrophoresis (1) and the fact that TSWV isolates maintained by repeated mechanical transmission may develop deletions in the G1G2 ORF (35).

Proteins with molecular masses of 58 and 95 kDa were produced in SF21 cells infected with TSWVGP and were detected with

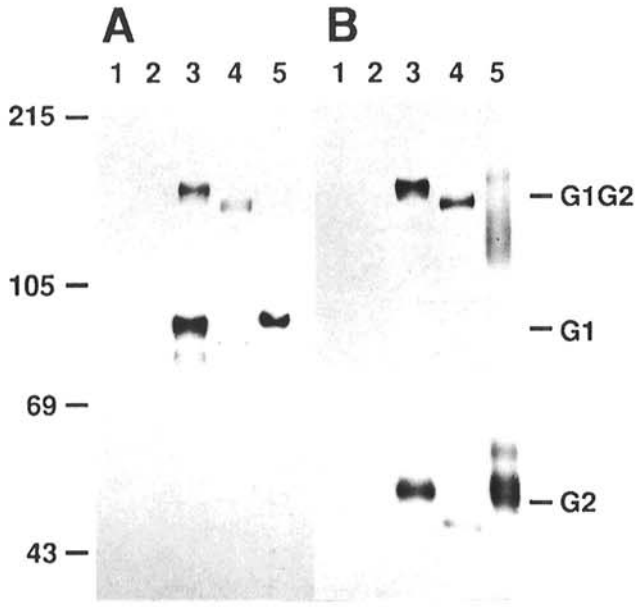

Fig. 2. Western blot analysis of proteins from recombinant baculovirus (TSWVGP)-infected *Spodoptera frugiperda* SF21 cells. Cell lysates (7×10^4 cell equivalents) were analyzed by electrophoresis on a 7.5% sodium dodecyl sulfate-polyacrylamide gel, transferred to Hybond C-Super nitrocellulose membranes (Amersham) and probed with a **A,** 1:100 dilution of anti-G1 or **B,** 1:275 dilution of anti-G2 monoclonal antibodies as described in text. A 1:25,000 dilution of rabbit anti-mouse immunoglobulin G conjugated to horseradish peroxidase (Sigma) and the ECL chemiluminescent system (Amersham) were used for detection. Lanes 1, 2, and 3, lysates of mock-, wild-type *Autographa californica* nuclear polyhedrosis virus-, and TSWVGP recombinant baculovirus-inoculated SF21 cells, respectively, harvested 72 h postinfection. Lanes 4, lysates from TSWVGP-infected cells grown in the presence of 25 µg of tunicamycin per ml. Lanes 5, isolated TSWV virions. The position of molecular weight markers (Life Technologies) (in kilodaltons) are indicated on the left. The positions of G1, G2, and the putative G1G2 polyprotein are indicated on the right.

the same pools of MAbs (Fig. 2A and B, lane 3), demonstrating expression from the G1G2 ORF and subsequent proteolytic cleavage of the G1G2 polyprotein in the absence of other TSWV proteins. This finding is consistent with the results of Kormelink (19) and the observation of cotranslational processing of the G1G2 polyprotein in several vertebrate-infecting viruses in the family Bunyaviridae in which the glycoproteins were expressed from purified G1G2 mRNAs (46,52) or cDNA via recombinant virus vectors (16,22,28,37,42). However, the high molecular mass protein (approximately 155 kDa) that reacted with both anti-G1 and -G2 MAbs in lysates of TSWVGP-infected SF21 cells (Fig. 2A and B, lane 3) is of an appropriate size to be the G1G2 polyprotein precursor, suggesting that proteolytic cleavage of the G1G2 polyprotein is not entirely cotranslational. Similar observations have been reported by Kormelink (19). The presence of multimeric forms of G1 and G2 also could explain the high molecular mass proteins because precedents for the formation of noncovalently linked G1-G2 heterodimers and G1 or G2 homodimers exist in the vertebrate-infecting viruses of the family Bunyaviridae (3,36). However, these dimers were resolved into monomers when analyzed under denaturing conditions (unless previously cross-linked) (3,36), making it likely that the high molecular mass protein observed in this study (Fig. 2A and B, lane 3) is the polyprotein precursor to G1 and G2. A protein in TSWV virion preparations with similar electrophoretic migration was detected only with anti-G2 MAbs (Fig. 2B, lane 5) and, therefore, could be a multimer of G2 that does not denature under the conditions of this experiment.

G1, G2, and their putative polyprotein precursor were N-glycosylated, as detected by their increased electrophoretic mobility when expressed in the presence of tunicamycin (Fig. 2A and B, lanes 3 and 4). The difference in the apparent molecular mass of G1 expressed in TSWVGP-infected SF21 cells and that found in TSWV virions (Fig. 2A, lanes 3 and 5) could be due to different patterns of glycosylation in insect and plant cells. Both plant and insect cells are capable of N-linked glycosylation, although some differences have been observed in the extent of their oligosaccharide side chain processing (26,45). The discrepancy between the 155-kDa putative precursor polyprotein observed in these experiments and the molecular mass predicted from the sequence of the G1G2 ORF (127 kDa) is likely due to glycosylation.

Glycosylation of the TSWV G1 and G2 proteins expressed in SF21 cells implies they have proceeded through normal translation and modification channels. Typically, proteins are translated on the rough ER, receive an oligosaccharide from a lipid intermediate, and then are transported to the Golgi complex for additional processing of the oligosaccharide prior to transport to their ultimate destination (15). Indirect immunofluorescent localization of TSWV G1 and G2 in TSWVGP-infected SF21 cells (Fig. 3) suggests a similar pattern of protein movement. Labeling of permeabilized SF21 cells with anti-G1 or -G2 MAbs was exclusively internal until some point between 24 and 48 h postinfection, at which time the label began to accumulate at the plasmalemma (Fig. 3B). Label continued to accumulate at the plasmalemma through 72 h postinfection (Fig. 3C). Support for the early internal location of G1 and G2 and the late external (plasmalemma) location was provided by labeling of G1 and G2 in nonpermeabilized SF21 cells (only labeling of external proteins was possible), in which no labeling was observed until 48 h postinfection (Fig. 3F). Movement of both TSWV G1 and G2 to and retention in the plasmalemma contrasts somewhat with the Golgi complex targeting generally observed for the glycoproteins of vertebrate-infecting viruses in the Bunyaviridae (16,22,27,28,33,39,47), although the plasmalemma localization of TSWV G1 and G2 observed in this study could be a reflection of the artificially high level of expression from the baculovirus system.

The experiments described in this paper provide evidence that *Tospovirus* glycoproteins can be proteolytically processed and glycosylated in the absence of other viral proteins. Whether heter-

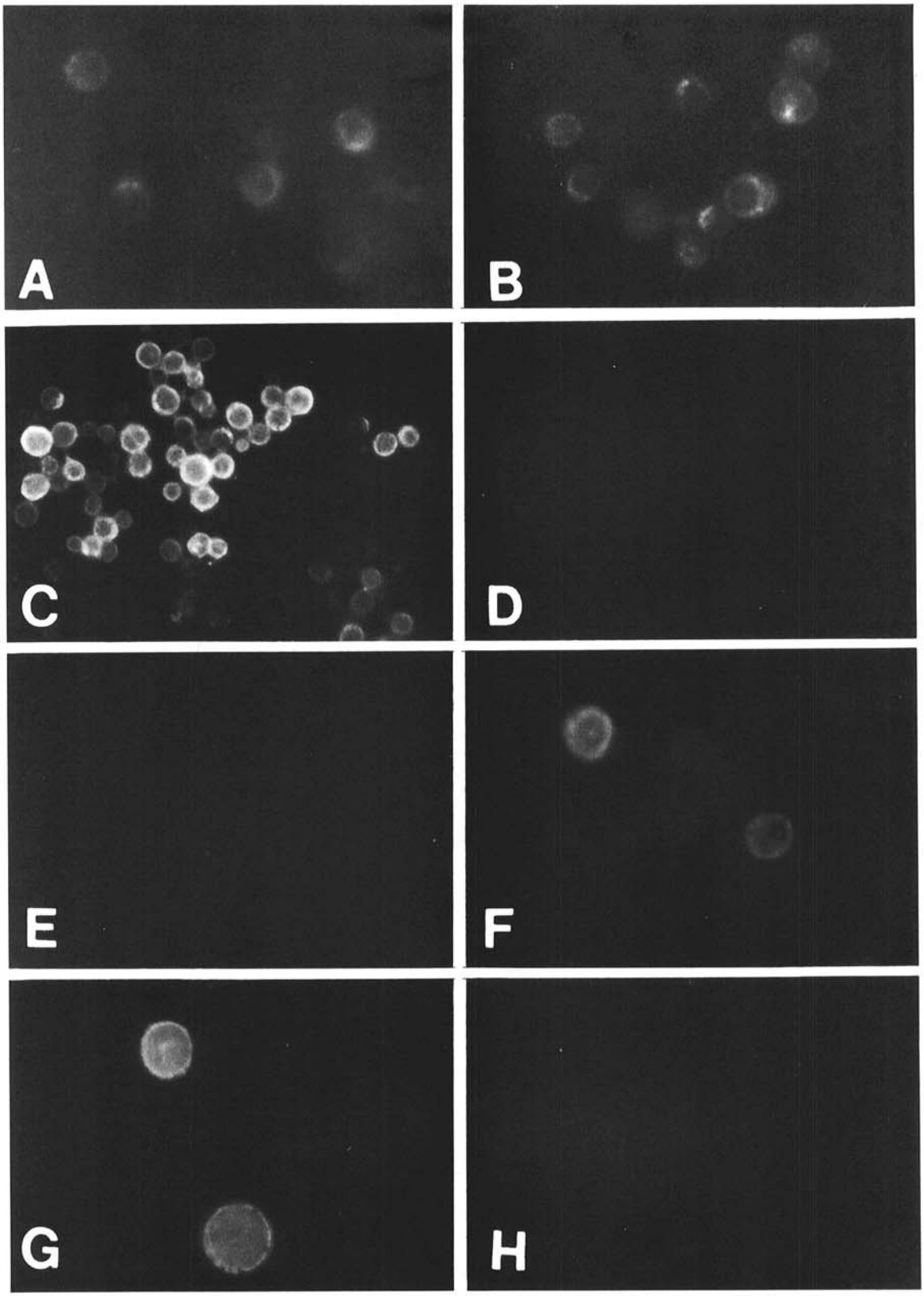

Fig. 3. Detection of tomato spotted wilt virus (TSWV) G1 expression in TSWVGP-infected *Spodoptera frugiperda* SF21 cells by indirect immunofluorescence. Monolayers of SF21 cells grown on glass coverslips were inoculated with TSWVGP, incubated at 27°C, and fixed with either a 1:1 mixture of methanol/acetone at **A,** 24, **B,** 48, or **C,** 72 h postinfection or 2% paraformaldehyde at **E,** 24, **F,** 48, or **G,** 60 h postinfection. Controls consisted of wild-type *Autographa californica* nuclear polyhedrosis virus-infected cells fixed 72 h postinfection with **D,** methanol/acetone or **H,** 2% paraformaldehyde. G1 was labeled with a 1:20 dilution of anti-G1 monoclonal antibodies, counterstained with a 1:200 dilution of goat anti-mouse immunoglobulin G conjugated to fluorescein isothiocyanate, and detected with ultraviolet radiation as described in text.

ologous expression of TSWV G1 and G2 has revealed a true difference in glycoprotein processing (posttranslational as oppcsed to cotranslational) between the plant- and vertebrate-infecting viruses of the family Bunyaviridae awaits additional experimentation. The proteolytic processing of glycoprotein precursors in the family Bunyaviridae is poorly understood. Although a host protease is assumed to cleave the glycoprotein precursors, neither the enzymes nor their cleavage sites within the polyprotein have been identified (41). Thus, the reagents developed for these experiments will be useful tools for further investigations of the mechanism of TSWV glycoprotein processing and the roles of these proteins in viral replication, maturation, and transmission.

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