Molecular biology of umbraviruses: phantom warriors

Michael E. Taliansky and David J. Robinson

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

The genomes of umbraviruses differ from those of most other viruses in that they do not encode a coat protein, and thus no virus particles are formed in infected plants. Protection of umbraviral RNA outside the host plant, during vector transmission, utilizes the coat protein of an assistor luteovirus, but this review focuses on the mechanisms that compensate for the lack of a coat protein in processes within the host plant. As well as an RNA-dependent RNA polymerase, umbravirus genomes encode two other proteins from almost completely overlapping open reading frames. One of these is a cell-to-cell movement protein that can mediate the transport of homologous and heterologous viral RNAs through plasmodesmata without the participation of a coat protein. The other, the ORF3 protein, binds to viral RNA to form filamentous ribonucleoprotein particles that have elements of helical structure. It serves to stabilize the RNA and facilitates its transport through the vascular system of the plant. It may also be involved in protection of the viral RNA from the plant’s defensive RNA-silencing response, although it is not a suppressor of silencing. The ORF3 protein also enters the cell nucleus, specifically targeting the nucleolus. Although the function of this localization is unknown, the ORF3 protein may provide a valuable tool for investigating plant nucleolar function.

INTRODUCTION

One of the main characteristics of most viruses is the formation of virus particles or virions, in which the viral genomic nucleic acid (RNA or DNA) is protected by encapsidation with one or more types of capsid (or coat) protein (CP). In virions, molecules of CP are packed into regular uniform structures based on either helical or icosahedral symmetry. In plant viruses, CP is also involved in transmission by biological vectors and often in the spread of viruses in infected plants. Some plant viruses, such as Cowpea mosaic virus (CPMV; Wellink & van Kammen, 1989), Potato virus X (PVX; Santa Cruz et al., 1998) or Cucumber mosaic virus (CMV; Canto et al., 1997), require CP for movement both through plasmodesmata (cell-to-cell movement) and via the phloem (long-distance movement). Others, such as Tobacco mosaic virus (TMV), require CP for long-distance movement but not for cell-to-cell movement (Carrington et al., 1996). Umbraviruses are distinguished from most other viruses by their lack of a gene for CP, and as a result these viruses do not form conventional virus particles.

The name of the genus Umbravirus is derived from the Latin umbra, which means shadow, both in the physical sense and in the metaphorical senses of a phantom or an uninvited guest who comes with an invited one. This name reflects the way in which umbraviruses depend for survival in nature on an assistor virus, which is always a member of the family Luteoviridae (referred to here as a ‘luteovirus’). For transmission between plants, CP of luteovirus forms aphid-transmissible hybrid virus particles, encapsidating umbraviral RNA (for review, see Taliansky et al., 2000). In nature, each umbravirus is associated with one particular luteovirus, although in experiments other luteoviruses can substitute for the natural assistor (Waterhouse & Murant, 1983; Cockbain et al., 1986). Transcapsidation, however, is not needed for umbravirus accumulation within infected plants because functions such as protection and movement of the virus RNA do not require the presence of the luteovirus or its CP (Demler et al., 1994). Moreover, under experimental conditions, mechanical transmission of umbraviruses can take place without the aid of an assistor virus, allowing them, like ‘phantom warriors’, to invade plants systemically without any CP and without producing virus particles. This implies that umbraviruses encode some product(s) that functionally compensate for the lack of a CP.

The genus Umbravirus comprises seven distinct virus species: Carrot mottle virus (CMoV), Carrot mottle mimic virus (CMoMV), Groundnut rosette virus (GRV), Lettuce speckles mottle virus (LSMV), Pea enation mosaic virus-2 (PEMV-2), Tobacco mottle virus (TMoV) and Tobacco bushy top virus (TBTV). Some of these viruses have been known since the early days of plant virology. The first to be described was...
Umbraviruses, and in particular their taxonomy, classification and evolution, and their biological properties, such as host range, pathology, geographical distribution, mechanisms of aphid transmission, interaction with assistor luteoviruses and satellite RNAs, have been reviewed in detail (Murant, 1993; Demler et al., 1996; Naidu et al., 1998; Robinson & Murant, 1999; Taliansky et al., 2000). In this article we will focus on new molecular findings that highlight the distinctive features of umbraviruses that follow from the absence of a capsid protein and virions.

**Genome organization, expression and replication**

The genomes of umbraviruses consist of one linear segment of positive-sense ssRNA. They are probably not polyadenylated at their 3′ ends (Halk et al., 1979); there is no information about structures at their 5′ ends. The complete genomic RNA sequences of CMoMV, GRV, PEMV-2 and TBTVM have been determined and are relatively short, comprising 4201, 4019, 4253 and 4152 nucleotides, respectively (Demler et al., 1993; Taliansky et al., 1996; Gibbs et al., 1996; Mo et al., 2003). Fig. 1 shows the genome organization of GRV (Taliansky et al., 1996); those of other umbraviruses are very similar (Demler et al., 1993; Gibbs et al., 1996; Mo et al., 2003). At the 5′ end, a very short non-coding region precedes ORF1, which encodes a putative 31–37 kDa protein. ORF2, which slightly overlaps the 3′ end of ORF1, might encode a 63–65 kDa protein but lacks an AUG initiation codon near its 5′ end. However, immediately before the stop codon of ORF1 there is a 7 nt sequence that is associated with frameshifting in several plant and animal viruses, and it seems probable that ORF1 and ORF2 are translated as a single 94–98 kDa polypeptide by a mechanism involving a −1 frameshift. The predicted amino acid sequence of the ORF2-encoded part of this protein has similarities with the sequences of the RNA-dependent RNA polymerases (RdRp) of viruses in the genera Carmovirus, Dianthovirus, Luteovirus, Machlomovirus, Necrovirus and Tombusvirus, and contains all eight conserved motifs of RdRp of positive-strand RNA viruses (Koonin & Dolja, 1993). Thus, it seems likely that the umbraviral ORF1/ORF2 fusion protein too is an RdRp. Since this enzyme is the only universally conserved protein of positive-strand RNA viruses, the genus *Umbravirus* might be considered to be in or close to the family Tombusviridae. It is not known whether translation of ORF1 without frameshifting into ORF2 also occurs.

A short untranslated region separates ORF2 from ORFs 3 and 4, which overlap each other almost completely in different reading frames and each yield a 26–29 kDa product (Fig. 1). The ORF4 product contains sequences characteristic of plant virus cell-to-cell movement proteins (MPs; see below). The ORF3 products encoded by different umbraviruses have up to 50 % sequence similarity to each other but no significant similarity to any other viral or non-viral proteins, and function to protect viral RNA and enable its transport through the phloem (see below). Two subgenomic RNA species derived from the 3′ end of the GRV genome have been detected, both of the appropriate size to be mRNAs for expression of ORF3 and/or ORF4 (Fig. 1; Taliansky et al., 1996). Thus, like other small RNA virus genomes that show economy in the use of coding sequences, the genome of umbraviruses is densely packed, with ORF3 overlapping much of ORF4, and with different types of expression mechanisms, including frameshifting and production

---

**Fig. 1.** The genome of GRV showing the different expression strategies: production of subgenomic RNA(s) [sgRNA(s)], initiation of translation at the first optimal AUG on the genomic RNA (gRNA; ORF1) or sgRNA(s) (ORF3 and ORF4), and initiation of translation as for ORF1 and frameshift (ORF1 + ORF2). Lines represent RNA molecules, grey boxes represent open reading frames and black boxes represent translation products.
of subgenomic RNAs (Fig. 1). As mentioned above, the four genomes whose complete sequences are known lack plausible ORFs for a CP.

Satellite RNAs are associated with some umbraviruses. In the case of GRV, satellite RNA is found in all naturally occurring isolates, and is primarily responsible for the symptoms of groundnut rosette disease (Murant et al., 1988; Murant & Kumar, 1990). GRV satellite RNA is an ssRNA of 895–903 nt, which relies on GRV for its replication (Blok et al., 1994) and, more unusually, is also required for the *Groundnut rosette assistor virus* (GRAV)-dependent aphid transmission of GRV (Murant, 1990). Thus, unlike most virus satellite RNAs, it is essential for the biological survival (though not the replication) of its helper virus. The role of the satellite RNA in the transmission process is to mediate transcapssidation of GRV RNA by GRAV protein to form stable aphid-transmissible hybrid virus particles (Robinson et al., 1999). Although different GRV satellite RNA variants contain up to five potential ORFs (Blok et al., 1994), none of the ORFs are essential for any of the functions and biological properties that have been ascribed to GRV satellites, including aphid transmission of GRV (Taliansky & Robinson, 1997; Robinson et al., 1999). In contrast, the satellite RNA that is associated with some isolates of PEMU-2 is not required for transcapssidation of PEMU-2 RNA by the CP of its assistor virus PEMU-1 or for aphid transmission of the hybrid particles (Demler et al., 1996b), and other umbraviruses, such as CMoV, do not have satellite RNAs, yet are transcapssidated by their assistors and thus transmitted by aphids. The reasons for these differences have not been explained.

Little information is available on the mechanisms of replication of umbravirus genomes. Experiments on replication in protoplasts have not been done, but absolute dependence of the replication of the satellite RNAs on an umbravirus helper has been demonstrated in whole plants (Murant et al., 1988; Murant & Kumar, 1990; Demler et al., 1994), although it does not have to be the natural helper (Demler et al., 1996a). Thus, the helper virus replicase is presumably involved in replication of satellite RNA.

**Cell-to-cell movement**

The spread of virus infection through a plant proceeds in two distinct phases: (i) cell-to-cell movement through plasmodesmata and (ii) long-distance movement through vascular tissues. It is generally accepted that cell-to-cell movement involves virus-encoded MPs as well as host-encoded components (Carrington et al., 1996). It has been shown that different MPs may facilitate cell-to-cell movement by different mechanisms. For some viruses, e.g. TMV, the MP interacts with plasmodesmata, increasing their size exclusion limit, and possesses non-specific RNA-binding activity that enables it to form a transportable complex with viral RNA (Wolf et al., 1989; Atkins et al., 1991; Citovsky & Zambryski, 1991). TMV MP has been shown also to bind to the cytoskeleton (Heinlein et al., 1995; McLean et al., 1995) and to microtubules (Boyko et al., 2000). Although TMV CP is essential for long-distance movement, it is not required for cell-to-cell movement (see review by Carrington et al., 1996). For other viruses, such as CPMV, the MP forms tubular structures extending through plasmodesmata of infected cells, and is believed to facilitate cell-to-cell spread of CPMV in the form of virions (Van Lent et al., 1990, 1991; Kasteel et al., 1997). CPMV therefore requires CP for cell-to-cell movement. For a growing number of viruses, it has been shown that MPs possess both the tubule-forming and the RNA-binding activities (Perbal et al., 1993; Jansen et al., 1998; Canto & Palukaitis, 1999), suggesting that both ‘virion’ and ‘non-virion’ mechanisms of cell-to-cell movement may co-exist and that viruses may use different mechanisms in different hosts or tissues. In contrast to viruses such as TMV and CPMV that produce a single MP, the genomes of some other viruses, such as PVX, contain a triple gene block that encodes three proteins required together with CP for cell-to-cell movement (Angell et al., 1996; Lough et al., 1998; Santa Cruz et al., 1998).

The ORF4-encoded proteins of umbraviruses have many characteristics of the MPs from other plant viruses. Firstly, they show significant sequence similarity to other MPs, in particular to the MP encoded by CMV (Taliansky et al., 1996). Secondly, like TMV MP, the GRV ORF4 protein localizes to plasmodesmata (Ryabov et al., 1998). Thirdly, this protein has been shown also to form tubular structures that protrude from the surface of protoplasts and to bind to RNA *in vitro* (Nurkiyanova et al., 2001). However, in contrast to other MPs that bind to RNA cooperatively, the GRV ORF4 protein binds to RNA incompletely and non-cooperatively (Nurkiyanova et al., 2001). Cooperative complexes containing viral RNA fully and tightly packaged by MP molecules are presumably necessary for cell-to-cell movement of, for example, TMV RNA but prevent the RNA molecules from being translated or replicated until they are released, probably by phosphorylation of the MP (Karpova et al., 1997). However, in the case of GRV ORF4 protein, the complex is not so densely packed and therefore the RNA may be available for translation or replication without the need for prior modification of the complex.

Finally, direct evidence for cell-to-cell movement activity of the ORF4 protein comes from gene replacement experiments. The ORF4 protein encoded by GRV was able to replace functionally the MPs of PVX (all the products encoded by the triple gene block; Ryabov et al., 1998) and CMV (the 3a MP; Ryabov et al., 1999a). Both PVX and CMV require their CP for cell-to-cell movement. However, the ORF4 protein enabled cell-to-cell movement of PVX and CMV regardless of the presence or absence of their CPs, although the CPs were still required for long-distance movement. All these results indicate that the umbravirus ORF4 proteins are cell-to-cell MPs and are adapted to transport viral RNAs, including the RNAs of unrelated viruses, without requiring any contribution from CP molecules.
Long-distance movement

Much less is known about long-distance virus movement. It is not clear how viruses enter, move through and exit from the vascular system, which is usually surrounded by bundle sheath cells and contains various cell types, including vascular parenchyma cells, companion cells and enucleate sieve elements (Nelson & van Bel, 1998; Opara & Turgeon, 1999; Santa Cruz, 1999). Thus, transport of a virus into and within vascular tissue implies movement from mesophyll cells to bundle sheath cells, from bundle sheath cells to vascular parenchyma and/or companion cells, and entry into sieve elements. Virus exit from vascular tissue presumably involves the same steps in reverse order. With only a few exceptions (Swanson et al., 2002), CP is essential for efficient long-distance transport of plant viruses; in the rare instances in which the CP gene is partially or wholly dispensable for systemic spread, the time required for systemic invasion is often increased (Cadman, 1962; Scholthof et al., 1993).

Once it became clear that umbraviruses do not encode a CP, it seemed possible that the ORF3-encoded protein, to which no function had yet been ascribed, might have a role in long-distance movement. CP is not required for cell-to-cell movement of TMV, but is essential for long-distance movement (Carrington et al., 1996). However, chimeric TMV derivatives, in which the CP gene was replaced by ORF3 of GRV, PEMV-2 or TMoV, were able to move rapidly through the phloem, confirming the role of the ORF3 protein in this process (Ryabov et al., 1999a, 2001). It is important to differentiate the umbravirus-encoded ORF3 proteins from other viral proteins, such as the 2b protein of CMV and the helper component-protease (HC-Pro) encoded by potyviruses, which also enhance systemic spread but do so by blocking an RNA-mediated host defence mechanism (akin to post-transcriptional gene silencing, PTGS). The 2b and HC-Pro proteins are suppressors of PTGS (Anandalakshmi et al., 1998; Brigneti et al., 1998; Kasschau & Carrington, 1998). However, attempts to demonstrate similar suppressor activity by the GRV or PEMV-2 ORF3 proteins, using experimental systems designed by Voinnet et al. (1999, 2000), were unsuccessful (our unpublished results; O. Voinnet, personal communication). Thus, the ORF3 proteins represent a novel class of long-distance movement factors (Ryabov et al., 1999b), which are able to transport unrelated viral RNAs that are not coated by a viral CP.

The umbravirus-encoded ORF3 protein is a multifunctional RNA-binding protein

Long-distance movement is not the only function of the umbravirus-encoded ORF3 protein. Another important function is the stabilization of viral RNA. In spite of the absence of a CP and conventional virus particles, umbraviral RNA accumulates to high levels in infected plants. Infectivity in water or buffer extracts from these plants survives for several hours at room temperature (Smith, 1945; Murant et al., 1969; Falk et al., 1979a; Reddy et al., 1985; Demler et al., 1994) and is resistant to treatment with ribonuclease (Murant et al., 1985). Subsequently, it was demonstrated that the ORF3 proteins encoded by PEMV-2, TMoV or GRV could increase the longevity of TMV RNA in lysates of protoplasts (Ryabov et al., 2001).

Thus, the umbravirus-encoded ORF3 proteins possess at least two functions, stabilization of viral RNA and mediation of its long-distance movement, that are usually characteristic of plant virus-encoded CPs. Both these functions suggest that the ORF3 proteins can interact with viral RNA. Indeed, analysis of the properties of recombinant GRV ORF3 protein expressed from TMV, a plant virus expression vector, in Nicotiana benthamiana plants showed that it bound to RNA cooperatively (Taliansky et al., 2003). The recombinant ORF3 protein also has a strong tendency to aggregate and forms stable dimers, trimers and higher order oligomers.

To understand how the ORF3 protein operates in infected cells and tissues to promote protection of viral RNA and its long-distance spread, the intracellular localization of the ORF3 protein was investigated. GRV ORF3 protein was expressed in N. benthamiana plants as a fusion with jellyfish green fluorescent protein (GFP) from heterologous virus gene vectors based on PVX and TMV (Ryabov et al., 1998). Confocal laser scanning microscopy of epidermal and mesophyll cells showed that, regardless of which gene vector was used, the ORF3-GFP fusion protein accumulated in cytoplasmic granules, some of which were associated with the large cytoplasmic inclusion bodies typical of PVX and TMV infections, whereas others (generally smaller ones) were distributed elsewhere in the cytoplasm, often in association with membranes (Fig. 2; Ryabov et al., 1998). These granules were apparently identical to the amorphous cytoplasmic inclusions that were detected by electron microscopy (Taliansky et al., 2003). These inclusions consisted of filamentous ribonucleoprotein (RNP) particles, embedded within an electron-dense matrix material (Fig. 3). The filaments were flexuous, non-rigid, structures with a diameter of 13–14 nm, and appeared to have an electron-lucent central hole about 4 nm in diameter. They had some elements of helical structure similar to the filamentous particles of viruses such as potyviruses or closteroviruses, but were not as uniform as classical virions. The apparently helical segments were interspersed with regions in which no regularity was discernible (Taliansky et al., 2003). Immunogold-labelling and in situ hybridization assays (Fig. 3) showed that the filamentous RNP particles contained viral RNA and the ORF3 protein. The inclusions were detected in all types of cells and were abundant in phloem-associated cells, in particular companion cells and immature sieve elements. Complexes similar in appearance to the RNP-containing inclusions were isolated from plants infected with TMV expressing GRV ORF3 or with GRV itself (Taliansky et al., 2003). It is suggested that the cytoplasmic structures containing the ORF3 protein serve to protect viral RNA, and that the filamentous RNP particles may be the
form in which it moves through the phloem. Thus, the RNP particles may be the umbravirus alternative to classical virions.

Formation of the cytoplasmic RNP complexes may also be involved in the protection of viral RNA from the plant’s defensive RNA-silencing response, which is based on the sequence-specific degradation of foreign, and in particular viral, RNA molecules. A key feature of the RNA-silencing pathway is the generation of dsRNA that corresponds in sequence to the target (virus) RNA. This dsRNA is cleaved into short interfering RNAs 21–25 nt in length and these are thought to mediate the target specificity for RNA degradation (for recent reviews, see Carrington, 2000; Vance & Vaucheret, 2001; Voinnet, 2001). To combat host defence RNA silencing, some plant viruses encode silencing suppressor proteins that also block PTGS in transgenic plants. Other viruses escape from the silencing defence reaction using other as yet unidentified mechanisms. No suppressor activity has been associated with umbraviruses and their encoded proteins, and in particular the ORF3 protein does not suppress PTGS (our unpublished results; O. Voinnet, personal communication), suggesting that umbraviruses may possess another mechanism to escape RNA silencing. One possible mechanism could be based on sequestration of viral RNA incorporated into cytoplasmic complexes, making it ‘invisible’ to the silencing machinery of the cell.

Earlier work identified membrane-bound, vesicle-like structures about 50 nm in diameter at the tonoplast and in
the vacuole in thin sections of cells infected with several umbraviruses (Murant et al., 1969, 1973; Falk et al., 1979b; Cockbain et al., 1986) and similar structures were seen in infective, clarified extracts (Murant et al., 1973; Reddy et al., 1985). Moreover, the infectivity of extracts was destroyed by treatment with ether or chloroform (Murant et al., 1969; Reddy et al., 1985). The relationship between these structures and the cytoplasmic inclusions observed by Ryabov et al. (1998) and Taliansky et al. (2003), and the role of lipid in the preservation of infectivity remain unclear.

Involvement of the nucleolus in umbravirus infection

The localization studies of the ORF3 protein mentioned above also provided another quite unexpected finding: in addition to the cytoplasmic granules, GFP-labelled ORF3 protein was also found in nuclei, preferentially but not exclusively targeted to nucleoli (Fig. 2; Ryabov et al., 1998).

The nucleolus is a prominent subnuclear domain and is classically regarded as the site of transcription of rRNA, processing of the pre-rRNAs and biogenesis of pre-ribosomal particles. However, in addition to these traditionally recognized nucleolar activities, the nucleolus also participates in many other aspects of cell function as well. Thus, because it is a site of transient sequestration and maturation of several factors and regulatory complexes, the nucleolus may be involved in the regulation of signal recognition particle biogenesis, small nuclear RNA processing, mRNA nuclear export, telomerase activity, the cell cycle, cell growth and ageing (for recent reviews see Lamond & Earnshaw, 1998; Pederson, 1998; Cockell & Gasser, 1999; Carmo-Fonseca et al., 2000; Olson et al., 2000).

A number of viruses interact with the nucleolus and its proteins. Certain viral proteins co-localize with, reorganize and re-distribute some nucleolar antigens such as nucleolin, B23 and fibrillarin (see review by Hiscox, 2002). Many of these interactions are not restricted to any particular type of virus, with examples found in retroviruses, DNA viruses and RNA viruses. It has been suggested that viruses may target the nucleolus and its components to favour viral transcription, translation and perhaps to alter cell growth and the cell cycle in order to promote virus replication (Hiscox, 2002). For example, the nucleoprotein (N protein) encoded by CMV (Mackenzie & Tremaine, 1988), the P3 protein with unknown function from Tobacco etch virus (a potyvirus; Langenberg & Zhang, 1997), and the CP of Tomato yellow leaf curl virus (a begomovirus; Rojas et al., 2001). In this last case, CP acts as a nuclear shuttle to traffic viral DNA into and out of the nucleus, which is the site of its replication. However, the specific involvement of the nucleolus remains obscure.

To target the nucleolus, a virus-encoded protein must first enter the nucleus by crossing the nuclear double-membrane envelope through the nuclear pore complex in an energy-dependent manner. Typically, proteins eligible for nuclear import contain specific nuclear localizing signals (NLS), which are characterized by one or two stretches of basic amino acids (see review by Nakielny & Dreyfuss, 1999). Database searches with the sequence of the umbraviral 26–29 kDa ORF3 proteins revealed no significant similarity with any other viral or non-viral proteins, except the corresponding proteins encoded by different umbraviruses (Taliansky et al., 1996), suggesting that there are no analogous proteins encoded by other viruses. This is perhaps not surprising if the ORF3 protein is the functional replacement for CP, which other viruses possess. Comparison of amino acid sequences of the umbraviral ORF3 proteins shows that they are rich in arginine, proline, serine and glycine residues. Measurement of local compositional complexity, using the SEG program (Wootton & Federhen, 1996), indicated that 85–95% of residues in each protein fall within predicted low-complexity, non-globular regions. Further analysis revealed that the most conserved central region of these proteins consists of a rather basic and highly hydrophilic domain (amino acids 108–130), which seems to be exposed on the protein surface and includes a highly basic arginine-rich sequence (positions 109–123) (Fig. 4) (Ryabov et al., 1999a) that resembles a NLS.

Some proteins that shuttle between the nucleus and cytoplasm also contain nuclear export signals (NES); a
prototypic leucine-rich NES has been found in the Rev protein of Human immunodeficiency virus type1 (Wen et al., 1995). Another conserved region (amino acids 151–180) of the umbravirus ORF3 protein is hydrophobic and contains invariant leucine residues in an LXXLL motif (Fig. 4), which resembles a NES. This putative NES may be conserved among the ORF3 proteins to ensure that they can be exported back to the cytoplasm and prevent their being trapped in the nucleus.

How proteins may be further delivered to the nucleolus is poorly understood. The nucleolus does not have apparent membrane or other barriers, and entry into it does not require energy, unlike entry to the nucleus. It has been suggested that viral proteins localize to the nucleolus by associating directly or indirectly, via a nucleolar localization signal or nucleolar retention signal, with nucleolar components, such as rDNA or its transcripts (Carmo-Fonseca et al., 2000; Hiscox, 2002). The conserved arginine-rich domain of the umbravirus ORF3 proteins is not unlike some of the nucleolar localization signals listed by Hiscox (2002). Thus, the presence of this sequence may explain the accumulation of ORF3 protein in the nucleolus. Like many host-encoded nucleolar proteins that exchange rapidly between nucleolus and nucleoplasm (Chen & Huang, 2001), ORF3 protein was also detected in the nucleoplasm, although at a lower concentration than in the nucleolus (Ryabov et al., 1998). This suggests that it too may shuttle into and out of the nucleolus, or that it is associated with a host protein that does so.

Although the umbraviral ORF3 proteins lack significant sequence similarities to proteins encoded by other viruses, some functional similarities may be noted. All viruses with negative-sense RNA genomes encode an RNA-binding nucleoprotein (NP) that encapsidates the virus genome to form RNP particles, which somewhat resemble the RNP particles formed by the umbravirus ORF3 proteins. However, the NP of Influenza A virus (a well-studied example) is much more than just a structural RNA-binding protein; it also functions as a key adaptor molecule between virus and host cell processes. Like the umbraviral ORF3 proteins, it is a nuclear shuttle protein and is involved in intracellular trafficking of the virus genome (Portela & Digard, 2002). Another protein encoded by Influenza A virus, the non-structural NS1 protein, also has a functional similarity to the umbraviral ORF3 proteins. NS1, like the ORF3 proteins, is a multifunctional protein, and both the ORF3 proteins and the NS1 protein protect viral RNA. Whereas the ORF3 proteins protect viral RNA from ribonuclease attack and possibly from the defensive RNA-silencing response of the plant, the NS1 protein blocks the alpha/beta interferon defensive system by binding and sequestering dsRNA, which is the inducer of the interferon system (Garcı´ a-Sastre, 2001). One may note that the ORF3 proteins also bind to dsRNA (Taliansky et al., 2003). The NS1 protein also targets the nucleus, where it plays a key role in the modulation of virus and host gene expression by blocking mRNA processing, splicing, polyadenylation and nuclear export (for example, see Salvatore et al., 2002), processes which may also be affected by the umbraviral ORF3 proteins.

The distribution of GFP-labelled GRV ORF3 protein within the nucleolus is not uniform (Ryabov et al., 1998) but resembles that of the granular component, which is the site
for the later stages of ribosome biogenesis (Beven et al., 1996). However, the ORF3 protein-associated RNP structures identified in the cytoplasm (see above) were not found in ultrathin sections of nuclei (Taliantsky et al., 2003). Thus the ORF3 protein in nuclei is apparently in a different form from that in the cytoplasm, and the role of its nuclear/nucleolar localization is still enigmatic. In particular, it is unclear whether ORF3 protein in the nucleus/nucleolus subsequently finds its way into the cytoplasmic RNP complexes, or whether the RNP complexes and the nucleolus are alternative destinations for the cytoplasmically synthesized protein. The likely pathways taken by ORF3 protein are illustrated in Fig. 5.

Concluding remarks and perspectives

The past few years have brought remarkable progress in our understanding of the genome organization and expression of umbraviruses, which differ from most other viruses by their lack of real virus particles. Virus genomes of four different umbraviruses have been completely sequenced, full-length cDNA clones giving infectious virus RNA transcripts have been generated, and functions of virus-encoded proteins have been identified. At the same time, the recent findings raise some new and fascinating questions. How do the cytoplasmic RNP complexes formed by the viral RNA and the ORF3 protein enter, pass through and then exit from the phloem? How and why does the umbravirus protein modify nucleolar activities? Does it interact with RNA components of the nucleolus, such as rRNAs or small nucleolar RNAs to modify RNA metabolism, or does it bind to one or more nucleolar proteins, inhibiting their enzymatic or other activities? Does the ORF3 protein have an effect on nucleolar sequestration of the cell growth and cell cycle regulators? Future research will address these questions and attempt to open up the ‘nucleolar black box’ in our understanding of the mechanisms of umbraviral infections. If the interaction of the ORF3 protein with nucleolar factors is related to its role in long-distance virus RNA movement, we would find that there may be a more general link between nucleolar functions and long-distance macromolecular transport in plants. Indeed, umbraviruses may serve as a powerful tool for testing hypotheses about nucleolar activity and function in plants.

ACKNOWLEDGEMENTS

This work was supported by a grant-in-aid from the Scottish Executive Environment and Rural Affairs Department. We thank Dr A. Musheghan for suggestions on computer analysis of the amino acid sequences described here. We also thank Dr E. Ryabov and Dr S. H. Kim for fruitful discussions.

REFERENCES


Molecular biology of umbraviruses

groundnut rosette disease complex and pea enation mosaic virus: sequence similarities and ability of each other’s helper virus to support their replication. J Gen Virol 77, 2847–2855.


