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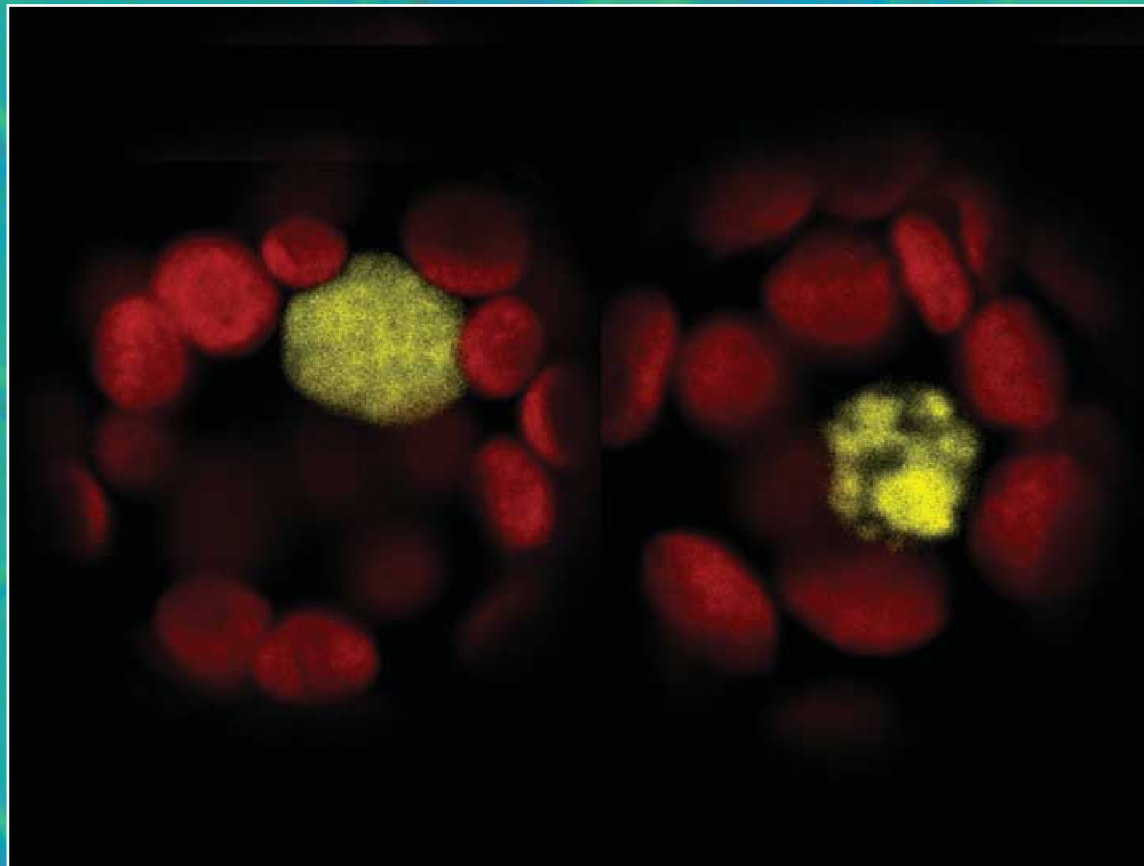

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Review

Nuclear import and export of plant virus proteins and genomes

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SUMMARY

Nuclear import and export are crucial processes for any eukaryotic cell, as they govern substrate exchange between the nucleus and the cytoplasm. Proteins involved in the nuclear transport network are generally conserved among eukaryotes, from yeast and fungi to animals and plants. Various pathogens, including some plant viruses, need to enter the host nucleus to gain access to its replication machinery or to integrate their DNA into the host genome; the newly replicated viral genomes then need to exit the nucleus to spread between host cells. To gain the ability to enter and exit the nucleus, these pathogens encode proteins that recognize cellular nuclear transport receptors and utilize the host's nuclear import and export pathways. Here, we review and discuss our current knowledge about the molecular mechanisms by which plant viruses find their way into and out of the host cell nucleus.

INTRODUCTION

Bidirectional substrate exchange between the cell nucleus and the cytoplasm is a selective, receptor-mediated process, which provides a crucial communication avenue within the cell. A number of animal and plant pathogens recognize the host's nuclear transport receptors and utilize cellular nuclear import mechanisms to gain access to the nucleus, which allows them to take advantage of the host DNA replication and repair machinery. An additional advantage to using the host nuclear import machinery is the ability to infect non-dividing cells, in which the integrity of the nucleus is maintained throughout their infection cycle.

Although most plant viruses replicate in the host cell cytoplasm, there are several large virus families, such as geminiviruses and caulimoviruses, which replicate in the nucleus. For all nucleus-replicating viruses, nuclear transport is a crucial stage in the infection cycle, which can be targeted to create novel antiviral compounds. In recent years, peptides and single-chain antibodies have been generated to impede nuclear import of the human immunodeficiency virus-1 (HIV-1) proteins Tat and Vpr, and thus inhibit viral replication (Krichevsky *et al.*, 2003, 2005). Success of this antiviral strategy, which can be applied to a variety of animal and plant viruses, emphasizes the importance of understanding the nuclear transport of viral proteins and genomes. Although traffic of animal viruses into and out of the host cell nucleus has been extensively reviewed (e.g. Cros and Palese, 2003; Smith and Helenius, 2004; Weidman *et al.*, 2003; Whittaker and Helenius, 1998; Whittaker *et al.*, 2000), the field of nuclear transport of plant viruses has remained largely out of the spotlight. Here, we remedy this lack of attention by reviewing our current knowledge of molecular mechanisms of nucleocytoplasmic transport of plant viral proteins and genomes.

CELLULAR PATHWAYS FOR NUCLEAR TRANSPORT

Before discussing nuclear import and export of plant viruses, it is important briefly to describe the general principles and major molecular participants in the process of nuclear transport in uninfected cells (Fig. 1). Nuclear import and export are based on a network of proteins that shuttle between the nucleus and the cytoplasm, allowing substrate exchange through nuclear pore complexes (NPCs), selective channels spanning the nuclear envelope (reviewed in Adam, 2001; Bagley *et al.*, 2000; Vasu and Forbes, 2001; Wozniak and Lusk, 2003). An NPC is a large proteinaceous structure (Fig. 1), which contains approximately 30 structural proteins (nucleoporins, Nups) in yeast (Rout *et al.*, 2000) and in vertebrates (Cronshaw *et al.*, 2002), and has a molecular mass ranging from 44–66 MDa in yeast (Rout and Blobel, 1993; Rout *et al.*, 2000; Yang *et al.*, 1998) to 60–125 MDa in vertebrates (Cronshaw *et al.*, 2002; Reichelt *et al.*, 1990). Studies of the NPCs in various organisms suggest that these structures

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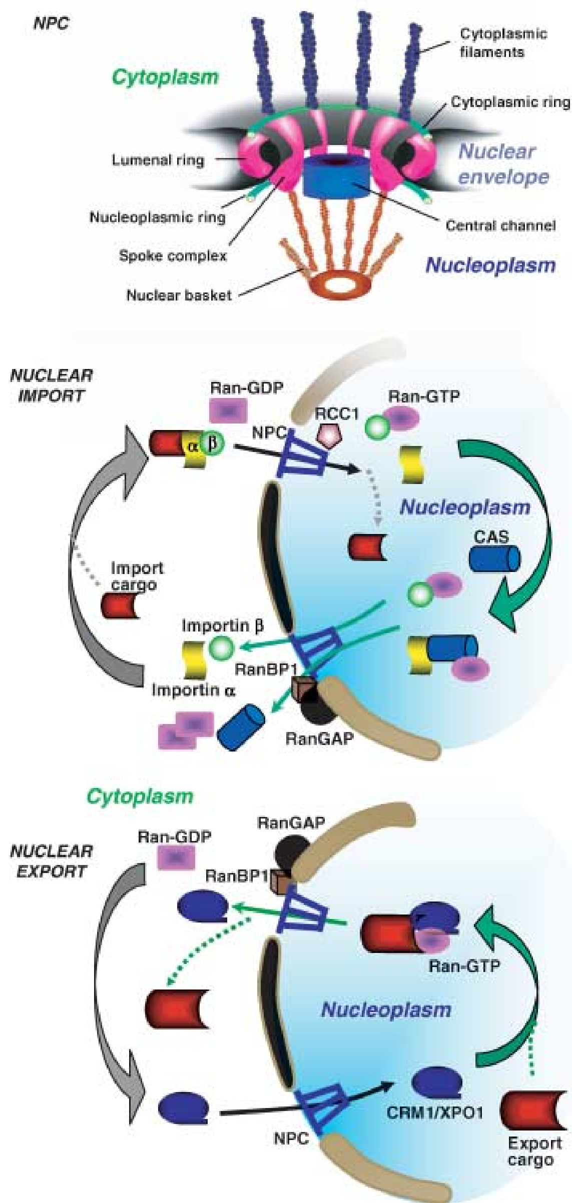

Fig. 1 Major steps of protein import and export through the nuclear pore complex (NPC). A schematic model of the NPC, largely derived from electron tomographic studies, represents a structure with an eight-fold rotational symmetry (not indicated here for simplicity) composed of a central channel, a spoke complex anchored to the nuclear envelope by the luminal ring, and cytoplasmic and nucleoplasmic rings, which are connected to the cytoplasmic filaments and nuclear basket, respectively. Nuclear import, illustrated for cargo molecules with a basic-type NLS, initiates in the cytoplasm with binding of the NLS of the cargo molecule to the importin α subunit of the importin α/β heterodimer; the resulting complex is transported to the NPC and, then, in the presence of Ran-GDP is translocated into the nucleoplasm. Within the nucleus, Ran-GDP is converted to Ran-GTP by RCC1, and Ran-GTP binds to importin β and dissociates the cargo/importin α/β complex. The cargo remains in the nucleus whereas the importin β/Ran-GTP complex is exported through the NPC, and importin α, following binding to its export factor CAS which binds Ran-GTP, is also exported as an importin α/CAS/Ran-GTP complex. On the cytoplasmic face of the NPC, Ran-GTP is hydrolysed to Ran-GDP by RanGAP, causing dissociation of the exported complexes and allowing recycling of the import factors. Nuclear export, illustrated for cargo molecules with a leucine-rich NES, begins in the nucleoplasm with binding of the NES of the cargo molecule to CRM1/XPO1 and Ran-GTP; the resulting complex exits the nucleus via the NPC, and, on the cytoplasmic face of the NPC, its dissociation is induced by Ran-GTP hydrolysis, which is facilitated by RanGAP and RanBP1.

are well conserved among eukaryotes. For instance, antibodies against the yeast nucleoporin Nsp1 recognize a 100-kDa protein in nuclear matrix preparations isolated from carrot suspension cells, suggesting that an Nsp1-like protein is an element of the NPC in plants (Cronshaw *et al.*, 2002; Meier, 2005; Scofield *et al.*, 1992; Vasu and Forbes, 2001).

Small molecules—up to 9 nm in diameter (Görlich and Kutay, 1999) or up to 70 kDa for globular proteins (Dingwall, 1991; Görlich and Kutay, 1999)—can enter the nucleus by simple diffusion. However, this diffusion is reasonably fast only for proteins of up to 20–30 kDa (Görlich and Kutay, 1999), and molecules and multimolecular complexes larger than 70 kDa [some complexes that traverse NPC are ribosomal subunits with a diameter of approximately 35 nm (reviewed in Meier, 2005)] must rely exclusively on active nuclear import. Furthermore, even some proteins that are smaller than 30 kDa, such as histones, are imported into the nucleus by an active mechanism (Breeuwer and Goldfarb, 1990; Jäkel *et al.*, 1999). The 'classical' active nuclear import pathway begins with a pair of nuclear import receptors—importin α and importin β (also known as karyopherin α and karyopherin β) (reviewed in Chook and Blobel, 2001; Goldfarb *et al.*, 2004; Görlich *et al.*, 1995b; Görlich, 1997; Meier, 2005; Powers and Forbes, 1994; Smith and Raikhel, 1999). In the cytoplasm, the importin α/β heterodimer binds to a protein containing nuclear localization signal (NLS) via the NLS-binding region of importin α. This trimeric complex is docked to the cytoplasmic face of the NPC and targeted to its core through the affinity of importin β for the NPC components (Chi *et al.*, 1995; Enenkel *et al.*, 1995; Görlich *et al.*, 1995a; Görlich, 1997; Kraemer *et al.*, 1995; Nigg, 1997; Radu *et al.*, 1995). Translocation into the nucleus requires recruitment of a small GTPase, Ran, in a GDP-bound form (Görlich, 1997). Once in the nucleus, dissociation of the complex containing both importins, Ran and the cargo protein is triggered by conversion of Ran-GDP to Ran-GTP by the Ran nucleotide exchange factor, regulator of chromosome condensation 1 (RCC1) (Görlich, 1997; Smith and Raikhel, 1999). Recent data indicate that the presence of Ran-GTP also allows large cargo molecules to translocate efficiently through central regions of the NPC (Lyman *et al.*, 2002). Finally, importins and Ran-GTP are recycled into the cytoplasm—where Ran-GTP is converted to Ran-GDP by the cytoplasmic Ran-GTPase activation protein (RanGAP) and

Ran-binding proteins (e.g. RanBP1), which enhance the normally low GTPase activity of Ran—and routed back to the nuclear import cycle (reviewed in Fried and Kutay, 2003; Komeili and O'Shea, 2001; Meier, 2005; Mosammaparast and Pemberton, 2004) (Fig. 1).

Aside from via the 'classical' import pathway, a number of proteins enter the nucleus by different mechanisms. Some proteins, such as Rev and Tat of HIV-1, do not require importin α for their nuclear import, but instead bind directly to importin β (Truant and Cullen, 1999). Yet another mechanism involves a homologue of importin β, called transportin, which binds to a specific 38-amino-acid-long sequence, termed M9, within the target proteins (Bonifaci *et al.*, 1997; Fridell *et al.*, 1997; Pollard *et al.*, 1996). Finally, other types of NLSs, such as the negatively charged NLS of the HIV-1 Vpr protein for which no obvious nuclear import pathways have been suggested (Karni *et al.*, 1998), imply the existence of additional pathways for nuclear import (Deane *et al.*, 1997; Görlich, 1997; Schlenstedt *et al.*, 1997).

Similar to nuclear import, substrates exported from the nucleus use targeting sequences, termed nuclear export signal (NES), and specific receptors that recognize them. Several nuclear export receptors, such as CRM1/exportin (XPO) 1 (Fornerod *et al.*, 1997; Stade *et al.*, 1997) and CAS (Kutay *et al.*, 1997), have been identified in different eukaryotic organisms. During the export process, Ran-GTP in the nucleus stimulates binding of CRM1 to the export substrate, and, after translocation through the NPC, the CRM1/Ran-GTP/cargo protein complex is disassembled following dissociation of Ran-GTP, which is promoted by RanBP1- and RanGAP-facilitated conversion of Ran-GTP to Ran-GDP on the cytoplasmic side of NPC. The exported molecule is released into the cytoplasm, CRM1 is recycled back to the nucleus and Ran-GDP is directed to the nuclear import cycle (reviewed in Fried and Kutay, 2003; Komeili and O'Shea, 2001; Kutay and Guttinger, 2005; Meier, 2005) (Fig. 1).

NUCLEAR IMPORT AND EXPORT SIGNALS

How does the nuclear import machinery of the cell identify the 'cargo' proteins that should be transported into the nucleus and distinguish them from those that should not? This task is achieved by recognition of specific protein sequences—the NLS—within the cargo protein. Usually, the NLS is a stretch of 7–20 amino acids within karyophilic cellular or viral proteins (Dingwall and Laskey, 1991; Garcia-Bustos *et al.*, 1991; Hicks and Raikhel, 1995; Nair *et al.*, 2003). Although there is no single consensus sequence for NLSs, they possess several common features. Typically, an NLS is rich in basic amino acids, not cleaved from the protein after import and functionally independent of its position within the protein molecule. One of the best studied examples of such basic-type NLS is the NLS of the large T antigen of simian virus 40 (SV40), represented by a single basic amino acid domain, PKKKRKV (Kalderon *et al.*, 1984a,b). Another well-studied class of basic-type NLSs are the bipartite signals, exemplified by the NLS of *Xenopus* nucleoplasmin KRPAATKKAGQAKKKK, which are composed of two short basic regions (underlined) separated by a peptide spacer (Dingwall and Laskey, 1991; Robbins *et al.*, 1991). Although the basic-type NLSs are by far the most common class of such signals, several other types of NLSs have been described, such as the NLS of Mat-α 2-type protein (Hall *et al.*, 1984), which contains a mix of hydrophobic and basic amino acids, or the negatively charged NLS of the HIV-1 Vpr protein (Friedler *et al.*, 1998).

Just as the nuclear entry of proteins requires an NLS, protein export out of the nucleus requires an NES (Kutay and Guttinger, 2005; Ossareh-Nazari *et al.*, 2001; Ullman *et al.*, 1997; Ward and Lazarowitz, 1999). NESs have been identified in a number of shuttling nuclear proteins from animals and viruses. First characterized in the HIV-1 Rev protein and the transcription factor TFIIIA from *Xenopus* (Fischer *et al.*, 1995; Fridell *et al.*, 1996; Wen *et al.*, 1995), the typical NES is, similarly to NLS, a relatively short segment of the protein, which is not cleaved from it after the export process has been completed and is functionally independent of its position within the protein sequence (Lazarowitz and Beachy, 1999). Sequence-wise, NESs are hydrophobic sequences of 10–13 amino acids, often rich in leucine residues, e.g. the NLS of HIV-1 Rev LQLPPLERLTL (Fischer *et al.*, 1995; Wen *et al.*, 1995), which are essential for the NES function (Fischer *et al.*, 1995; Fridell *et al.*, 1996; Wen *et al.*, 1995). In plant viruses, one of the well-described NESs is the NES of the *Squash leaf curl virus* (SLCV) nuclear shuttle protein (NSP, see also below), which is strikingly similar in its overall characteristics to other eukaryotic and viral NESs (Ward and Lazarowitz, 1999). Moreover, the *Xenopus* TFIIIA NES can functionally substitute for the SLCV NSP NES in both protein nuclear export and viral infectivity, suggesting that the basic nuclear export machinery is highly conserved between animals and plants (Ward and Lazarowitz, 1999).

PLANT-SPECIFIC CHARACTERISTICS OF THE NUCLEAR TRANSPORT MACHINERY

Studies of nuclear transport in animal, yeast and plant cells have revealed that the fundamental trafficking machinery is highly conserved (Corbett and Silver, 1997; Haizel *et al.*, 1997; Hicks and Raikhel, 1995; Merkle *et al.*, 1996). For instance, Ran homologues of *c.* 75% identity to Ran proteins found in vertebrates and fungi have been identified in Arabidopsis (Haizel *et al.*, 1997), tomato (Ach and Gruissem, 1994), tobacco (Merkle *et al.*, 1994) and other plant species (Smith and Raikhel, 1999). The requirement for GTP hydrolysis during nuclear import is conserved as well, because nuclear import in both vertebrates and plants can be blocked by non-hydrolysable GTP analogues (Görlich, 1997; Hicks *et al.*, 1996; Merkle *et al.*, 1996; Zupan *et al.*, 1996). Additionally, a lectin wheat germ agglutinin (WGA)

specifically binds to a subset of NPC proteins modified with a single *O*-linked β-*N*-acetylglucosamine (O-GlcNAc) residue and blocks nuclear import in permeabilized cultured animal cells (Dabauvalle *et al.*, 1988; Davis, 1995). Similarly, WGA recognizes glycoproteins in plant NPCs and blocks nuclear import of large molecules/macromolecular complexes in plant cells (Zupan *et al.*, 1996), but not in permeabilized plant protoplasts (Heese-Peck *et al.*, 1995; Hicks *et al.*, 1996; Merkle *et al.*, 1996), suggesting potential differences in nuclear import and/or variability in glycosylation of the NPC proteins between intact and permeabilized plant cells.

Despite its extensive similarity to other eukaryotic systems, nuclear import in plants may possess several unique features. First, nuclear import—inhibited at low temperatures in animal cells (Adam *et al.*, 1990)—occurs at 4 °C in plants (Hicks *et al.*, 1996; Merkle *et al.*, 1996), suggesting an evolutionary adaptation to growth in colder climates. Moreover, different plant species may have evolved different mechanisms for nuclear import. While nuclear import of the basic-type NLS-containing proteins in rice (and, potentially, other monocotyledonous plants) is mediated by the importin α/β heterodimer (Jiang *et al.*, 1998a; Jiang *et al.*, 1998b; Matsuki *et al.*, 1998), in *Arabidopsis thaliana* (and most likely other dicots), nuclear import is promoted by importin α alone, without the requirement for importin β. Indeed, the Arabidopsis genome does not encode a true homologue of importin β that functions in an importin α-dependent import pathway as in yeast and animals (Harel and Forbes, 2004). Thus, Arabidopsis importin α [AtI MPα (Hicks *et al.*, 1996) or AtKAPα (Ballas and Citovsky, 1997)], although homologous in its sequence to importins α of other, non-plant eukaryotes, probably differs from them functionally. Consistent with this idea, AtI MPa binds NLSs with high affinity (Hicks *et al.*, 1996; Hubner *et al.*, 1999; Smith *et al.*, 1997), whereas the NLS-binding affinity of importins α from yeast and mouse increases when they are complexed with importin β (Efthymiadis *et al.*, 1997; Hu and Jans, 1999; Hubner *et al.*, 1997; Rexach and Blobel, 1995). Intriguingly, recent studies in animal systems indicate that animal importins α can also function without importin β (Kotera *et al.*, 2005), although this importin β-independent activity may not represent the main function of importin α in animal cells. Unlike for nuclear import, there is no evidence that nuclear export machinery of plant cells substantially deviates from that in animal and yeast cells.

NUCLEAR TRANSPORT OF PLANT VIRAL PROTEINS INVOLVED IN NUCLEOCYTOPLASMIC TRAFFIC OF VIRAL GENOMES

DNA viruses: geminiviruses

Geminiviruses are a family of plant viruses, which draw their name from the morphology of the virus particles with twin icosahedral morphology (Lazarowitz, 1992; Timmermans *et al.*, 1994). Geminiviruses are currently classified in the family Geminiviridae, which comprises three genera, *Begomovirus*, *Mastrevirus* and *Curtovirus*—with a fourth genus, *Topocuvirus*, proposed—according to their genome, host plants and insect vector (Rybicki *et al.*, 2000; Stanley *et al.*, 1999). The geminivirus genome is composed of either two (bipartite geminiviruses) or a single (monopartite geminiviruses), covalently closed, circular, 2.5- to 2.9-kb-long single-stranded (ss) DNA molecule (Lazarowitz, 1992).

Because geminiviruses encode only a small number of proteins assisting in their replication (Elmer *et al.*, 1988; Hanley-Bowdoin *et al.*, 1990; Sunter *et al.*, 1990), and there is no evidence that these viral proteins function as DNA polymerases (Nagar *et al.*, 1995), to propagate they must take advantage of the replication machinery of the host cell, which requires access to the host cell nucleus. To gain nuclear access, geminiviruses have evolved to encode specific proteins, which contain NLS and NES signals, and are able to mediate transport of the viral DNA genomes in and out of the nucleus. Although all geminiviruses utilize this general strategy for nuclear transport of their genomic DNA, the nature of these viral nuclear shuttle proteins differs between bipartite and monopartite geminiviruses.

Bipartite geminiviruses

The bipartite genome of begomoviruses, such as SLCV and *Bean dwarf mosaic virus* (BDMV), contains two ssDNA molecules designated DNA A and DNA B. Most of the viral genes are located on the DNA A molecule (Elmer *et al.*, 1988), and the DNA B molecule contains only two genes that encode viral movement proteins: MPB, movement protein encoded by DNA B, formerly BL1 or BC1; and NSP, nuclear shuttle protein, formerly BR1 or BV1 (Sanderfoot and Lazarowitz, 1995; Sanderfoot *et al.*, 1996). MPB associates with the cell wall and membrane fractions of the cell, and it has been suggested to function to increase the exclusion limit of plasmodesmata, thereby facilitating cell-to-cell movement of the virus (Pascal *et al.*, 1993). By contrast, NSP shuttles between the nucleus and the cytoplasm, presumably mediating nuclear import of the viral genome (Sanderfoot and Lazarowitz, 1995; Sanderfoot *et al.*, 1996).

Both NSP and MPB bind single-stranded nucleic acids. However, in the case of SLCV, NSP binds ssDNA much better than MPB (Pascal *et al.*, 1994) whereas, in the case of BDMV, MPB binds double-stranded (ds) DNA and NSP binds both ssDNA and dsDNA with comparable affinity (Rojas *et al.*, 1998). Thus, SLCV MBP and NSP probably translocate the true viral genomic ssDNA (Pascal *et al.*, 1994) while BDMV MPB and NSP may mediate nuclear and/or cell-to-cell transport of a replicative dsDNA form of the virus (Noueiry *et al.*, 1994; Rojas *et al.*, 1998).

SLCV NSP was first shown to shuttle between the nucleus and the cytoplasm in transient expression assays in both insect cells

and tobacco protoplasts (Sanderfoot and Lazarowitz, 1995; Sanderfoot *et al*., 1996). When expressed alone, NSP is detected in the nuclei of transfected tobacco protoplasts; however, when co-expressed with MPB, it is redirected from the nucleus to the cortical cytoplasm, suggesting that MPB interacts with NSP and traps it at the cell periphery (Lazarowitz and Beachy, 1999; Sanderfoot and Lazarowitz, 1995, 1996). The NSP–MPB interaction was confirmed in the studies showing that the C-terminus of NSP contains a domain that directly binds MPB (Sanderfoot and Lazarowitz, 1995; Sanderfoot *et al*., 1996). Therefore, the current model, based on studies of SLCV and BDMV, suggests that MPB and NSP act in a cooperative manner to move the viral genome from the nucleus to the cytoplasm and across the cell wall through the plasmodesmal channels (Lazarowitz and Beachy, 1999; Sanderfoot and Lazarowitz, 1995; Sanderfoot *et al*., 1996). In particular, the mechanism of SLCV propagation probably involves the following steps: following initial infection, NSP transports the viral ssDNA into the host cell nucleus, or at least to the nuclear pore, for transcription, replication and subsequent assembly; NSP would bind some of the replicated viral genomes and bring them back out to the cytoplasm, where MPB traps NSP–ssDNA complexes and directs them through plasmodesmata into adjacent, uninfected cells (Lazarowitz and Beachy, 1999; Sanderfoot and Lazarowitz, 1996; Sanderfoot *et al*., 1996; S. Lazarowitz, personal communication). In these neighbouring cells, the MPB/NSP–ssDNA complexes dissociate, and NSP again assists in importing the viral genome into the nucleus to initiate new rounds of replication and infection (Lazarowitz and Beachy, 1999; Sanderfoot and Lazarowitz, 1995, 1996; Sanderfoot *et al*., 1996).

Not unexpectedly, NSP has been shown to contain two NLSs, both of which are required for NSP functionality and located in the N-terminal half of the protein: a bipartite NLS located between residues 22 and 41, and an SV40 T antigen-type NLS between residues 89 and 93 (Lazarowitz and Beachy, 1999; Sanderfoot *et al*., 1996). Consistent with its function in nuclear export of the viral genomes (see above), NSP has been shown to contain also an NES signal (Ward and Lazarowitz, 1999). A series of C-terminal truncations of NSP was fused to the β-glucuronidase (GUS) reporter gene and co-expressed with MPB in a plant cell. The rationale behind these experiments was that fusion proteins with an intact NSP C-terminal domain, which interacts with MPB, would be relocalized from the nucleus to the cortical cytoplasm only if they contained an NES (Ward and Lazarowitz, 1999). These studies identified an amino acid sequence required for the export of NSP from the nucleus, which resembles NESs found in other rapidly shuttling nuclear proteins. Mutation of the leucine residues in this sequence abolished both the SLCV infectivity and the relocation of NSP from the nucleus to the cortical cytoplasm of tobacco protoplasts in the presence of MPB, confirming its function in the nuclear export of the NSP (Ward and Lazarowitz, 1999).

One of the challenges faced by NSP is to balance between viral replication and nuclear export of the newly synthesized viral genomes. For SLCV and *Cabbage leaf curl virus* (CLCV), this process has been proposed to be controlled by interactions between NSP, the plant-specific nuclear acetyltransferase AtNSI (*Arabidopsis thaliana* NSP interactor) (McGarry *et al*., 2003) and the viral coat protein (CP) (Carvalho and Lazarowitz, 2004; McGarry *et al*., 2003). In this model, the invading geminiviral ssDNA genomes are converted by host nuclear enzymes into dsDNA minichromosomes, which serve as templates for transcription and for rolling-circle replication (Bisaro, 1996; Pilartz and Jeske, 1992). The newly replicated viral ssDNA molecules then are bound by CP, sequestered away from replication, and are made available for interaction with NSP (Qin *et al*., 1998; Sanderfoot *et al*., 1996). NSP recruits AtNSI which acetylates CP, decreasing its affinity for ssDNA, thus leading to CP displacement and formation of the NSP–ssDNA complexes competent for nuclear export (Carvalho and Lazarowitz, 2004; McGarry *et al*., 2003).

Monopartite geminiviruses

Monopartite geminiviruses lack the DNA B component of its genome as well as obvious MPB or NSP homologues, which may reflect fundamental differences in the mechanism of nucleocytoplasmic shuttling and cell-to-cell movement of monopartite vs. bipartite geminiviruses (Navot *et al*., 1991). Most of our knowledge about nuclear import and export of monopartite geminiviruses derives from studies of *Maize streak virus* (MSV) and *Tomato yellow leaf curl virus* (TYLCV).

MSV is a mastrevirus (Bock, 1999), which, similarly to other geminiviruses, must enter the host cell nucleus for replication and virus assembly, and then exit the nucleus for cell-to-cell movement. To this end, the MSV genome encodes two proteins, the movement protein (MP) (Mullineaux *et al*., 1988) and the CP (Morris-Krsinich *et al*., 1985), which are required for systemic infection (Boulton *et al*., 1989, 1993; Lazarowitz *et al*., 1989) and are involved in the intra- and intercellular transport of the viral genome. Specifically, MSV CP binds ssDNA and dsDNA (Liu *et al*., 1997), localizes to the nucleus within insect cells and tobacco protoplasts, and mediates nuclear import of viral DNA in maize and tobacco cells (Liu *et al*., 1999). As expected, MSV CP contains an NLS that is located at the N-terminus of the protein (Liu *et al*., 1997, 1999).

Unlike CP, MSV MP is a non-karyophilic protein the involvement of which in viral cell-to-cell movement is inferred from its association with plasmodesmata in infected tissues (Dickinson *et al*., 1996) and from the observations that GFP-tagged MSV MP translocates from cell to cell when expressed in maize leaf epidermis (Kotlizky *et al*., 2000). Interestingly, unlike NSPs of bipartite geminiviruses, MSV MP was unable to bind viral DNA, but, similarly to NSPs, it interacted with MSV CP and prevented, or strongly diminished, nuclear accumulation of MSV CP–DNA complexes in plant cells (Liu *et al*., 2001).

In the case of TYLCV, a begomovirus (Czosneck, 1999), both nuclear import and export of its genome are most probably mediated by a single viral protein, the CP (Kunik *et al.*, 1998; Rojas *et al.*, 2001). TYLCV CP binds ssDNA and is actively transported into the nucleus of plant (Kunik *et al.*, 1998; Rojas *et al.*, 2001) and insect cells (Kunik *et al.*, 1998). Using truncated TYLCV CP–GUS fusions, an NLS was identified within the N-terminal part of CP (Kunik *et al.*, 1998). CP nuclear import was inhibited by an excess of a peptide corresponding to the bipartite NLS of the *Agrobacterium* VirD2 protein, which competitively binds to importin α, as well as by GTPγS (a non-hydrolysable GTP homologue), suggesting that CP utilizes an importin α-dependent pathway for nuclear import (Kunik *et al.*, 1998). Indeed, an importin α protein, designated LeKAPα, from tomato (*Lycopersicon esculentum*) was identified and shown to interact directly with TYLCV CP (Y. Gafni, unpublished observation). This interaction is illustrated in Fig. 2, which shows that CP of the Israeli isolate of TYLCV directly binds LeKAPα in a living plant cell, and that the interacting proteins accumulate within the plant cell nucleus. In this study, the protein–protein interaction and subcellular localization were monitored by the bimolecular fluorescence complementation assay (BiFC) (Hu and Kerppola, 2003; Hu *et al.*, 2002; Lacroix *et al.*, 2005; Li *et al.*, 2005; Tzfira *et al.*, 2004).

In addition to carrying an NLS, TYLCV CP has been shown to contain a functional leucine-rich NES within the C-terminal part of the protein (Rhee *et al.*, 2000). Consistent with this nucleo-cytoplasmic shuttle activity, TYLCV CP has been shown to mediate nuclear import and export of fluorescently labelled DNA in plant cells (Rojas *et al.*, 2001). Therefore, in the current model for TYLCV intracellular transport, CP is thought to mediate the uptake of viral DNA into the host cell nucleus and, following replication, export the newly synthesized TYLCV genomes into the cell cytoplasm for subsequent cell-to-cell movement. Thus, TYLCV CP carries out at least two distinct functions, nucleo-cytoplasmic shuttling of viral genomes and encapsidation of the TYLCV DNA into viral particles, playing a vital role in the TYLCV infection cycle. Unlike its nuclear transport, cell-to-cell movement of TYLCV is not well characterized. This process has been proposed to be mediated, via an as yet unknown mechanism, by the viral C4 protein (Rojas *et al.*, 2001, 2005), which induces the disease symptoms (Jupin *et al.*, 1994; Krake *et al.*, 1998; Rigden *et al.*, 1994) and localizes to the host cell periphery and interacts with plasmodesmata (Rojas *et al.*, 2001, 2005). However, unlike MSV MP and CP, which interact with each other (Liu *et al.*, 2001), TYLCV C4 is unable to bind TYLCV CP (Y. Gafni, unpublished observation).

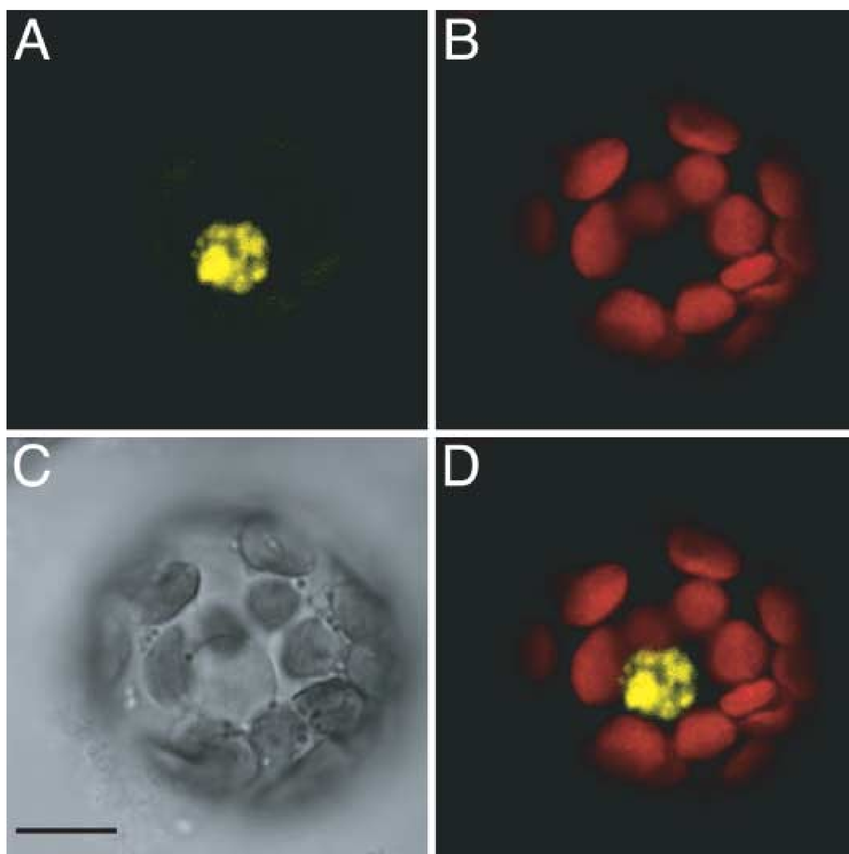

Fig. 2 TYLCV CP interacts with tomato karyopherin α (LeKAPα) in the nucleus of tobacco protoplasts. Protein–protein interaction and subcellular localization was assayed by bimolecular fluorescence complementation (BiFC) between cYFP-tagged CP and nYFP-tagged LeKAPα. (A) YFP signal. (B) Plastid autofluorescence. (C) Phase image. (D) Merged YFP and plastid autofluorescence signals. All fluorescence images are projections of several confocal sections. Scale bar = 10 μm.

RNA viruses: rhabdoviruses

The Rhabdoviridae family includes both animal and plant viruses that are characterized by distinctive bullet-shaped enveloped particles (reviewed in Jackson *et al*., 2005). Plant-infecting rhabdoviruses have been classified into two genera (Jackson *et al*., 2005; Tordo *et al*., 2005): Cytorhabdoviruses, such as *Lettuce necrotic yellows virus* (LNYV), are similar to the animal rhabdoviruses in that they replicate in the cytoplasm of infected cells, whereas nucleorhabdoviruses, such as *Sonchus yellow net virus* (SYNV), replicate in the host cell nucleus. Although the overall particle morphology and genome organization of SYNV (Jackson and Christie, 1979) is similar to that of cytoplasmically replicating animal rhabdoviruses, such as vesicular stomatitis virus (VSV) and rabies virus, unlike its animal counterparts, SYNV maintains its genomic RNA in the nucleus (reviewed in Jackson *et al*., 2005).

Three SYNV proteins, polymerase (L), nucleocapsid (N) and phosphoprotein (P), are present in the infectious viral core (Rose and Whitt, 2001; Wagner and Jackson, 1997). The N and P proteins represent two classes of essential nucleocapsid components that are found in all monopartite negative-strand RNA viruses and provide several vital functions during infection. The N protein encapsidates the full-length genomic and viral messenger RNAs as the nascent molecules are transcribed, as well as nascent leader RNAs during the transition period between transcription and replication (Wagner, 1987). The P protein has been shown to serve as a chaperone for several viral proteins (Masters and Banerjee, 1988; Takacs and Banerjee, 1995; Takacs *et al*., 1993), and its phosphorylation state has been implicated in the regulation of viral transcription (Gao *et al*., 1996) and replication (Das *et al*., 1997; Hwang *et al*., 1999).

NLS sequences have been identified in both N and P proteins of SYNV. Specifically, the N protein contains a bipartite NLS in its C-terminus (Goodin *et al*., 2001). In contrast, the P protein does not contain a classical basic amino acid residue-rich NLS and does not bind importin α *in vitro*; instead, nuclear import of the P protein is directed by an NLS within its N-terminal domain (Goodin *et al*., 2001). In addition, a putative NES was suggested to be located in the C-terminus of the P protein (Martins *et al*., 1998), indicating that this viral protein shuttles between the nucleus and the cytoplasm of the host cell; indeed, when expressed alone, P is detected both in the cell nucleus and in the cytoplasm, whereas N is exclusively nuclear (Goodin *et al*., 2001, 2002). Interestingly, N and P proteins interact with each other, and their co-expression in plant and yeast cells results in a dramatic shift of both proteins to a distinct subnuclear, but not nucleolar (Tsai *et al*., 2005), location (Goodin *et al*., 2001, 2002). Recently, relocalization to a subnuclear locus, identified as nucleolus, was demonstrated for co-expressed N and P proteins of another nucleorhabdovirus, *Maize fine streak virus* (MFSV) (Tsai *et al*., 2005). Although cytorhabdoviruses also encode N and P homologues (Rose and Whitt, 2001), the intercellular fate of these proteins remains unknown, and it represents a focus of several ongoing studies (M. Goodin and R. G. Dietzgen, personal communication).

The presence of different types of NLSs in the SYNV core proteins suggests that this virus may utilize several different pathways for its nuclear import. This situation is reminiscent of the nuclear import of lentiviruses, such as HIV-1, in which several proteins in the infectious viral core, e.g. Vpr, integrase and matrix protein, carry different types of NLSs (Bukrinsky and Haffar, 1999). The presence of multiple NLSs of different types, which use different nuclear import receptors and pathways, might be explained by the necessity to effectively infect different types of cells or cells at different stages of development, when individual import pathways work with varying degrees of effectiveness.

The current model of SYNV infection suggests that, during early stages of infection, the viral core containing the N, P and L proteins and the SYNV genomic RNA is transported into the nucleus for replication of the viral genome and subsequent maturation of the virions (Jackson, 1978; Martins *et al*., 1998; Wagner *et al*., 1996), most probably utilizing the NLSs of the N and P proteins. Following viral replication, nucleorhabdovirus cores reassembly and bud from the intact or modified inner membrane of the nuclear envelope into perinuclear spaces; this budding most probably results in envelopment of the virus (Christie *et al*., 1974; Jackson, 1987; Jackson *et al*., 2005). In addition, SYNV nucleocapsid cores have been proposed to exit the nucleus and move cell to cell with the help of the viral movement protein, such as sc4 of SYNV (Jackson *et al*., 2005). It is tempting to speculate that this putative nuclear export of the SYNV cores is mediated by the viral P protein, which contains an NES signal (Martins *et al*., 1998).

It is of note that this peculiar mechanism of viral nuclear exit by budding from the inner nuclear membrane into the perinuclear space was also reported for herpesviruses (Leuzinger *et al*., 2005). Herpes simplex virus 1 (HSV-1) is an enveloped virus that infects animal cells by fusing with their plasma membranes and injecting its nucleoprotein capsid into the host cell cytoplasm (Spear, 1993). The HSV-1 capsid then docks at the NPC with the help of importin β, potentially in an importin α-independent fashion (Ojala *et al*., 2000), and releases the viral genome into the nucleoplasm (Batterson *et al*., 1983; Ojala *et al*., 2000; Roizman and Sears, 1996). HSV-1 exit out of the nucleus and envelopment probably occur via two distinct pathways: budding of capsids at the inner nuclear membrane into the perinuclear space and egress of capsids through impaired nuclear pores, followed by budding at the outer nuclear membrane or at membranes of the rough endoplasmic reticulum or Golgi (Leuzinger *et al*., 2005).

Plant pararetroviruses: caulimoviruses

Cauliflower mosaic virus (CaMV) is the type member of the caulimoviruses (Shepherd, 1981), one of the six genera in the family

Caulimoviridae (Pringle, 1999). Its genome consists of a circular dsDNA (Frank *et al.*, 1980; Hull and Covey, 1985; Shepherd, 1981, 1989) packaged by the viral CP into an icosahedral particle with a diameter of approximately 54 nm (Cheng *et al.*, 1992).

With regard to its replication mechanism, CaMV is a pararetrovirus because it employs reverse transcription in its infection cycle (Pfeiffer and Hohn, 1983; Rothnie *et al.*, 1994), but its genome is composed of DNA rather than RNA of true retroviruses (Rothnie *et al.*, 1994). CaMV replicates by first transcribing its genomic DNA to a polycistronic 35S RNA and then reverse transcribing the latter back to the genomic DNA (reviewed in Hull and Covey, 1985; Ryabova *et al.*, 2002; Shepherd, 1989). In addition, unlike retroviruses, which integrate their proviral DNA into the host genome (Brown, 1989), the genomic DNA of pararetroviruses, i.e. animal hepadnaviruses and plant caulimoviruses, accumulates within the nucleus of the infected cell as circular minichromosomes (Ménissier *et al.*, 1983; Newbold *et al.*, 1995; Olszewski and Guilfoyle, 1983; Tuttleman *et al.*, 1986). Whereas many retroviruses, such as murine leukaemia virus, can infect host cells only during cell division when the nuclear envelope is compromised (Roe *et al.*, 1993), pararetroviruses and the retroviral subgroup of lentiviruses, i.e. HIV-1 and HIV-2 (Hirsch *et al.*, 1995), developed means to import their genetic material into the host cell nucleus, enabling them to infect non-dividing, resting cells (Bukrinsky, 2004; Bukrinsky and Haffar, 1999; Bukrinsky *et al.*, 1993; Cullen, 2001; Izaurralde *et al.*, 1999; Karsies *et al.*, 2002).

Early in the replication cycle, CaMV imports its genomic DNA into the host cell nucleus, where it is assembled into minichromosomes (Ménissier *et al.*, 1983; Olszewski and Guilfoyle, 1983). From these minichromosomes, viral 35S RNA is produced and transported to the cytoplasm for translation and reverse transcription (Olszewski *et al.*, 1982; Pfeiffer and Hohn, 1983), where it contributes to establishment of a cytoplasmic pool of CaMV particles, which are then transported to other cells, taken up by the insect vectors or reinfect the nucleus (Hull and Covey, 1985).

Although the replication of CaMV has been a subject of extensive studies for several decades, information regarding the mechanism of nuclear import of viral DNA and proteins is just emerging. A functional basic amino acid residue-rich NLS was identified within the N-terminus of the CaMV CP (Leclerc *et al.*, 1999). This sequence was required for targeting of CaMV CP to the nuclei of plant protoplasts, and it was conserved in several other caulimoviruses (Leclerc *et al.*, 1999). This karyophilic nature of CaMV CP, combined with the presence of a nucleic acid binding domain in the C-terminal part of CP (Guerra-Peraza *et al.*, 2000), suggests that this protein may function to bind and transport the viral DNA into the host cell nucleus. Interestingly, nuclear import of CaMV CP is negatively regulated in *cis* by its acidic N-terminal extension; only the mature form of CP, in which the N-terminal extension has been removed by a CaMV-encoded protease (Karsies *et al.*, 2001; Torruella *et al.*, 1989), can enter the host cell nucleus (Karsies *et al.*, 2002; Leclerc *et al.*, 1999). This regulation of CP nuclear import, potentially due to masking of the NLS within the CP precursor, may be important for the viral infection cycle, e.g. to allow virus assembly in the cytoplasm of the infected cell (Karsies *et al.*, 2002; Leclerc *et al.*, 1999).

Consistent with the basic nature of its NLS, CaMV CP interacts with importin α (Karsies *et al.*, 2002), indicating that its nuclear import occurs by an importin α-dependent pathway. Lending support to the possible role of CP in nuclear targeting of CaMV, purified CaMV particles docked at the periphery of plant nuclei in cell-free plant systems (Karsies *et al.*, 2002). This docking was inhibited by classical inhibitors of nuclear import, anti-NLS antibodies and GTPγS (Karsies *et al.*, 2002). The docked CaMV virions, however, were unable to enter the nucleus (Karsies *et al.*, 2002); potentially, this lack of nuclear import is due to the large size of CaMV particles (*c.* 54 nm, Cheng *et al.*, 1992), which exceeds the diameter of particles known to be actively transported through the NPC (26–39 nm, Dworetzky and Feldherr, 1988; Feldherr *et al.*, 1984; Panté and Kann, 2002). Thus, after docking of the virions at the NPC, their capsids must either become physically distorted or, at least partially, disassemble and the DNA imported into the nucleus with the help of CP subunits (and/or another protein such as reverse transcriptase) still attached to the viral genome (Karsies *et al.*, 2002).

The mechanism by which nuclear import of CaMV in plant cells is effected and controlled is not specific for plant pararetroviruses, and it is employed by their animal hepadnavirus counterparts. Specifically, nuclear import of hepatitis B virus (HBV) is mediated by its core protein, which is presumed to carry a basic-type NLS (Kann *et al.*, 1999); this import process may be further assisted by the HBV reverse transcriptase, which also contains an NLS (Kann *et al.*, 1997). The activity of the HBV core protein NLS is negatively regulated by masking in immature core protein; however, unlike CaMV CP NLS, which is activated by proteolysis (Karsies *et al.*, 2001, 2002; Leclerc *et al.*, 1999; Torruella *et al.*, 1989), the HBV core protein NLS is unmasked upon phosphorylation (Kann *et al.*, 1999). Phosphorylated viral cores then dock at the NPC in an importin α/β-dependent fashion (Kann *et al.*, 1999). That both plant and animal pararetroviruses have evolved to regulate negatively their CP/core protein NLSs suggests the importance of this mechanism for the infection cycle, e.g. to ensure virus assembly in the host cytoplasm and to minimize the damage that these viral proteins could cause in the cell nucleus (Karsies *et al.*, 2002).

Unlike that of nuclear import, the role of nuclear export in CaMV biology is still unexplored. Because CaMV DNA is produced in the host cell cytoplasm by reverse transcription of the 35S RNA (Pfeiffer and Hohn, 1983), the viral infection cycle most likely does not require the reactions of DNA nuclear export. Instead, the CaMV 35S RNA may be exported into the host cell cytoplasm via the endogenous pathway(s) of mRNA nuclear

export (Erkmann and Kutay, 2004; Zenklusen and Stutz, 2001). Alternatively, by analogy with HIV-1 Rev-mediated nuclear export of the unspliced viral RNA (Fischer *et al.*, 1994), nuclear export of CaMV 35S RNA may also involve viral factors for which the identity remains to be determined. Regardless of its molecular mechanism, CaMV nuclear export is followed by intercellular transport through plasmodesmata, either as whole viral particles or as partially encapsidated genomes; this cell-to-cell movement of CaMV is mediated by its MP encoded by gene 1 of the virus (reviewed in Waigmann *et al.*, 2004).

NUCLEAR TRANSPORT OF PLANT VIRAL PROTEINS NOT INVOLVED IN NUCLEOCYTOPLASMIC TRAFFIC OF VIRAL GENOMES

It is important to emphasize that not all viral proteins that are imported into the host cell nucleus participate in the transport of viral genomes. Nuclear import of viral proteins also is involved in other stages of the viral infection cycle, such as suppression of the RNA silencing defence response of the host plant. Antiviral RNA silencing response represents the innate immune system of the plant involved in plant defence against viruses (Baulcombe, 2002a, 2004; Voinnet, 2001). In turn, viruses have evolved to encode RNA silencing suppressor proteins that counteract this host defence (Baulcombe, 2002b, 2004). Some of such viral suppressor proteins are known to localize to the host cell nucleus. Specifically, AC2 or transcriptional activator protein (TrAP)—an RNA silencing suppressor of a bipartite geminivirus, *Mungbean yellow mosaic virus-Vigna* (MYMV)—localizes to the cell nucleus via a basic-type NLS, which is required for the suppressor activity (Trinks *et al.*, 2005). In the case of a monopartite geminivirus TYLCV-China, silencing suppression has been attributed to its C2 protein (Dong *et al.*, 2003; van Wezel *et al.*, 2001, 2003), which represents a homologue of the AC2/TrAP proteins of bipartite geminiviruses (Hartitz *et al.*, 1999). C2 contains an arginine-rich NLS, and nuclear localization of C2 was suggested to be required for the C2-induced suppression of RNA silencing (Dong *et al.*, 2003) through an as yet unknown mechanism. Unlike nuclear replicating geminiviruses, *Cucumber mosaic virus* (CMV) is an RNA virus that replicates in the host cell cytoplasm (Palukaitis and Garcia-Arenal, 2003), yet its RNA silencing suppressor, the 2b protein (Brigneti *et al.*, 1998), is targeted to the cell nucleus (Mayers *et al.*, 2000) by one (Lucy *et al.*, 2000) or two arginine-rich NLSs (Wang *et al.*, 2004), which are recognized by a plant importin α (Wang *et al.*, 2004). Note that not all viral RNA silencing suppressors are nuclear proteins, and such viral suppressors as P19 of *Tomato bushy stunt virus* (TBSV), HcPro of *Cowpea aphid-borne mosaic virus* (CABMV) and γb of *Poa semilatent virus* (PSLV) reside outside of the nucleus (Mlotshwa *et al.*, 2002; Uhrig *et al.*, 2004; Yelina *et al.*, 2005).

Other proteins of cytoplasmically replicating viruses are also found in the cell nucleus. For example, nuclear inclusion a and b proteins (NIa and NIb) of potyviruses, such as *Tobacco etch virus* (TEV) and *Pepper vein banding virus* (PVBV), which function in the host cell cytoplasm, are imported into the nucleus via their basic-type NLSs, presumably for sequestration as aggregates (Anindya and Savithri, 2004; Carrington *et al.*, 1991; Li and Carrington, 1993; Li *et al.*, 1997; Schaad *et al.*, 1996). In addition, the 27-kDa protein, which is encoded by ORF3 of *Groundnut rosette virus* (GRV) and required for viral RNA protection and movement through the phloem, contains an arginine-rich NLS and a leucine-rich NES, and is located within the cell nucleus, preferentially targeting nucleoli (Ryabov *et al.*, 1998, 2004). Yet another viral protein, p25, encoded by RNA-3 of *Beet necrotic yellow vein virus* (BNYVV) and involved in symptom formation, is a nucleocytoplasmic shuttle that contains a basic-type NLS that interacts with plant importins α and a hydrophobic residue-rich NES (Vetter *et al.*, 2004). Similarly to these plant RNA viruses, cytoplasmically replicating animal RNA viruses also can transport their proteins, such as the protease-polymerase precursor 3CD of poliovirus, into the host cell nucleus (Weidman *et al.*, 2003).

Finally, the matrix (M) protein of SYNV is another viral protein capable of nuclear import (Goodin *et al.*, 2002). M is an inner component of the virion that connects the viral envelope to the ribonucleocapsid core, and, unlike the SYNV core proteins N and P (see above), M does not appear to participate directly in nuclear transport of the viral genome.

SUMMARY AND CONCLUSIONS

Nuclear import of viral genomes for replication and their subsequent export for cell-to-cell movement represent central stages in infection cycles of several members of major families of plant viruses. Thus, it is important to understand the mechanisms by which these nuclear transport processes occur, especially with regard to the viral factors that mediate nucleo-cytoplasmic shuttling of the invading viral genomes and the components of the host cell machinery that recognize these viral transport proteins. Our knowledge about plant viral nuclear import and export discussed in this review is summarized in Fig. 3, which depicts major events in nuclear import of bipartite and monopartite geminiviruses, rhabdoviruses and pararetroviruses, exemplified by SLCV, TYLCV, SYNV and CaMV, respectively. Although all of these viruses utilize the host cell machinery for nuclear transport, they vary in their strategies of harnessing this cellular pathway. Specifically, SLCV and SYNV have evolved to encode dedicated transport proteins, i.e. NSP and MPB for SLCV and N and P for SYNV, that bind viral genomes and direct them into and out of the nucleus. CaMV and TYLCV, by contrast, appear to be more biologically frugal, utilizing their CPs not only to encapsidate the

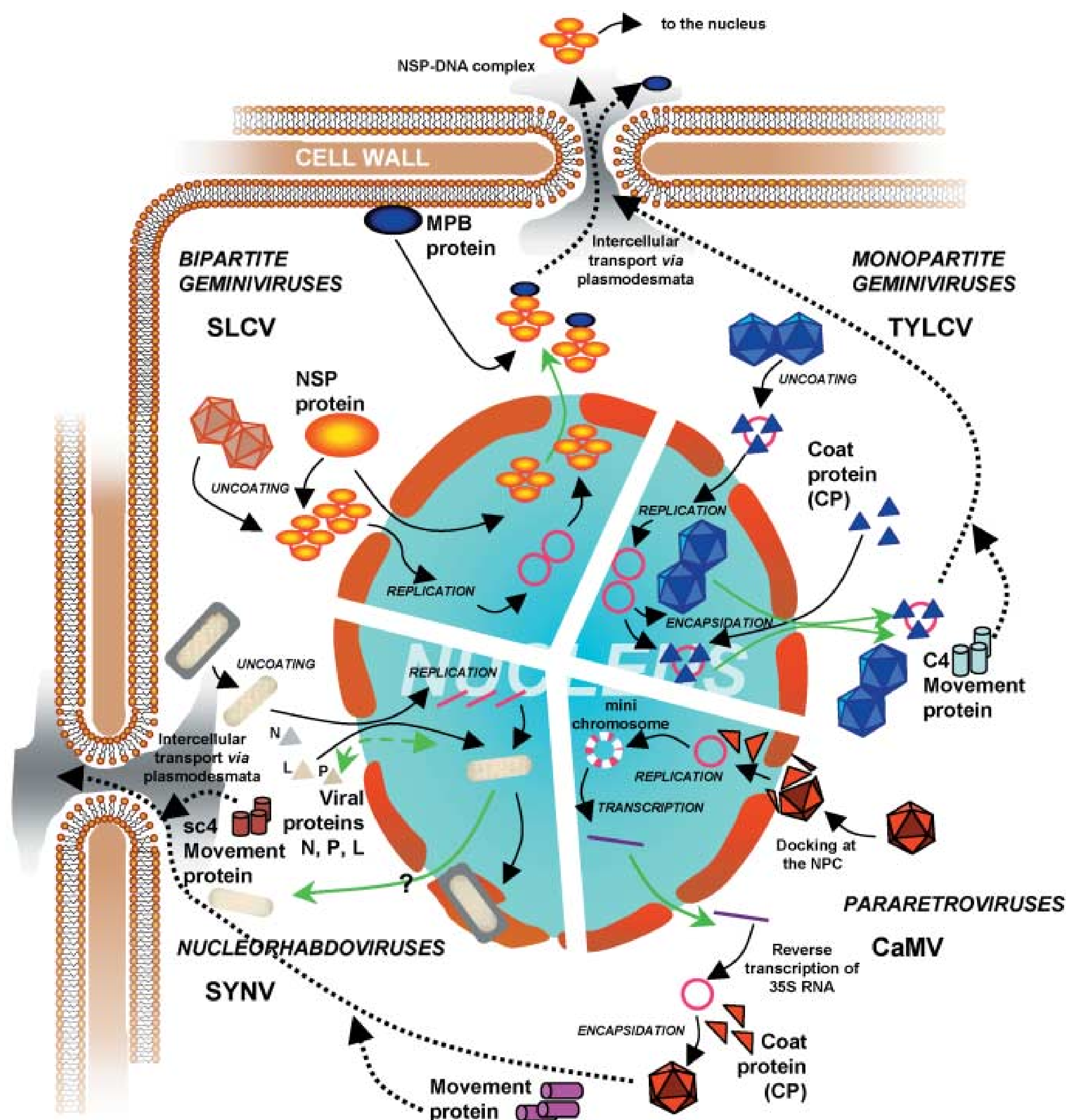

Fig. 3 Summary of nuclear import and export mechanisms of plant viruses belonging to the families of geminiviruses (bipartite and monopartite), nucleorhabdoviruses and pararetroviruses. Bipartite geminiviruses, exemplified by SCLV (*Squash leaf curl virus*), import their circular ssDNA genomes into the nucleus using the viral NSP (nuclear shuttle protein); following replication, the viral genomes are shuttled out of the nucleus by NSP, which then interacts with the viral MPB (movement protein encoded by DNA B) to move via plasmodesmata to the neighbouring cell. Monopartite geminiviruses, exemplified by TYLCV (*Tomato yellow leaf curl virus*), import their circular ssDNA genomes into the nucleus using the viral CP (coat protein), which most likely also participates in nuclear export of fully assembled virions or partially encapsidated genomes; the exported viral genomes probably move through plasmodesmata with the help of the viral C4 protein. Nucleorhabdoviruses, exemplified by SYNV (*Sonchus yellow net virus*), import their viral core composed of the linear ssRNA genome and L (polymerase), N (nucleoprotein) and P (phosphoprotein) proteins into the nucleus using the N protein; following replication, the core complexes reassembly and envelope by budding through the inner nuclear membrane into the perinuclear space; also, the cores may export from the nucleus with the help of the P protein and move from cell to cell with the help of the sc4 movement protein. Pararetroviruses, exemplified by CaMV (*Cauliflower mosaic virus*), import their circular dsDNA genomes from partially disassembled virions into the nucleus using the viral CP; within the nucleus, the genomic DNA is converted to minichromosomes, transcribed and the transcripts are exported into the cytoplasm, where the complete viral 35S RNA is reverse-transcribed to produce genomic DNA, which is encapsidated by CP and moves through plasmodesmata as complete or partially assembled virions with the help of the CaMV movement protein. SCLV and TYLCV are shown as twinned icosahedral particles (brown and blue, respectively), SYNV virion is depicted as a membrane envelope (grey rectangle) surrounding the viral core (light brown rod) and CaMV is shown as a single icosahedral particle (red). Viral genomic DNA or RNA are shown in red as circular or linear structures. All other viral components are indicated by their adjacent labels. NPCs are indicated as breaks in the nuclear envelope. Plasmodesmata are shown as membrane-lined channels with traversing endoplasmic reticulum (ER) indicated in grey. Arrows indicate the sequence of depicted events. For further details, see text.

genomic DNA molecules, but also to transport them into (and, in the case of TYLCV CP, also out of) the host cell nucleus.

Identification and characterization of the viral proteins and their targeting signals required for nuclear transport of plant viruses is important not only for better understanding of the viral infection cycles and infection mechanisms, but also for designing new antiviral strategies for crop protection. Indeed, many nuclear-replicating plant viruses represent major crop pathogens; for example, TYLCV is a major tomato pathogen and causes extensive crop losses world-wide (Gafni, 2003; Moriones and Navas-Castillo, 2000; Nakhla and Maxwell, 1997). Interfering with nuclear transport of such viral pathogens, e.g. by RNAi silencing of viral nuclear shuttle proteins or by competing with them using over-expression of their mutants with inactivated NLS and/or NES signals, would represent an attractive biological approach to prevent or attenuate viral infection of agronomically important plant species.

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REFERENCES

Ach, R.A. and Gruissem, W. (1994) A small nuclear GTP-binding protein from tomato suppresses a *Schizosaccharomyces pombe* cell cycle mutant. *Proc. Natl Acad. Sci. USA*, **91**, 5863–5867.

Adam, S.A. (2001) The nuclear pore complex. *Genome Biol.* **2**, reviews 0007.0001–0007.0006.

Adam, S.A., Marr, R.S. and Gerace, L. (1990) Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. *J. Cell Biol.* **111**, 807–816.

Anindya, R. and Savithri, H.S. (2004) Potyviral NIa proteinase, a proteinase with novel deoxyribonuclease activity. *J. Biol. Chem.* **279**, 32159–32169.

Bagley, S., Goldberg, M.W., Cronshaw, J.M., Rutherford, S. and Allen, T.D. (2000) The nuclear pore complex. *J. Cell Sci.* **113**, 3885–3886.

Ballas, N. and Citovsky, V. (1997) Nuclear localization signal binding protein from *Arabidopsis* mediates nuclear import of *Agrobacterium* VirD2 protein. *Proc. Natl Acad. Sci. USA*, **94**, 10723–10728.

Batterson, W., Furlong, D. and Roizman, B. (1983) Molecular genetics of herpes simplex virus. VIII. further characterization of a temperature-sensitive mutant defective in release of viral DNA and in other stages of the viral reproductive cycle. *J. Virol.* **45**, 397–407.

Baulcombe, D.C. (2002a) RNA silencing. *Curr. Biol.* **12**, R82–R84.

Baulcombe, D.C. (2002b) Viral suppression of systemic silencing. *Trends Microbiol.* **10**, 306–308.

Baulcombe, D.C. (2004) RNA silencing in plants. *Nature*, **431**, 356–363.

Bisaro, D.M. (1996) Geminivirus replication. In *Replication in Eukaryotic Cells* (DePamphilis, M., ed.), pp. 833–854. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Bock, K.R. (1999) *Maize streak virus.* CMI/AAB Description of Plant Viruses no. 133. URL: http://www.dpvweb.net/dpv/showdpv.php?dpvno=133

Bonifaci, N., Moroianu, J., Radu, A. and Blobel, G. (1997) Karyopherin beta2 mediates nuclear import of a mRNA binding protein. *Proc. Natl Acad. Sci. USA*, **94**, 5055–5060.

Boulton, M.I., Pallaghy, C.K., Chatani, M., MacFarlane, S. and Davies, J.W. (1993) Replication of maize streak virus mutants in maize protoplasts: evidence for a movement protein. *Virology*, **192**, 85–93.

Boulton, M., Steinkellner, H., Donson, J., Markham, P.G., King, D.I. and Davies, J.W. (1989) Mutational analysis of the virus-sense genes of maize streak virus. *J. Gen. Virol.* **70**, 2309–2323.

Breeuwer, M. and Goldfarb, D.G. (1990) Facilitated nuclear transport of histone H1 and other small nucleophilic proteins. *Cell*, **60**, 999–1008.

Brigneti, G., Voinnet, O., Li, W.X., Ji, L.H., Ding, S.W. and Baulcombe, D.C. (1998) Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*. *EMBO J.* **17**, 6739–6746.

Brown, P.O. (1989) Intergration of retroviral DNA. In *Retroviruses*, Vol. 157 (Swanstrom, R. and Vogt, P.K., eds), pp. 19–48. Berlin: Springer-Verlag.

Bukrinsky, M. (2004) A hard way to the nucleus. *Mol. Med.* **10**, 1–5.

Bukrinsky, M.I. and Haffar, O.K. (1999) HIV-1 nuclear import: in search of a leader. *Front. Biosci.* **4**, 772–781.

Bukrinsky, M.I., Haggerty, S., Dempsey, M.P., Sharova, N., Adzhubel, A., Spitz, L., Lewis, P., Goldfarb, D., Emerman, M. and Stevenson, M. (1993) A nuclear localization signal within HIV-1 matrix protein that governs infection of non-dividing cells. *Nature*, **365**, 666–669.

Carrington, J.C., Freed, D.D. and Leinicke, A.J. (1991) Bipartite signal sequence mediates nuclear translocation of the plant potyviral NIa protein. *Plant Cell*, **3**, 953–962.

Carvalho, M.F. and Lazarowitz, S.G. (2004) Interaction of the movement protein NSP and the *Arabidopsis* acetyltransferase AtNSI is necessary for Cabbage leaf curl geminivirus infection and pathogenicity. *J. Virol.* **78**, 11161–11171.

Cheng, R.H., Olson, N.H. and Baker, T.S. (1992) Cauliflower mosaic virus: a 420 subunit (T=7), multilayer structure. *Virology*, **186**, 655–668.

Chi, N.C., Adam, E.J. and Adam, S.A. (1995) Sequence and characterization of cytoplasmic nuclear protein import factor p97. *J. Cell Biol.* **13**, 265–274.

Chook, Y.M. and Blobel, G. (2001) Karyopherins and nuclear import. *Curr. Opin. Struct. Biol.* **11**, 703–715.

Christie, S.R., Christie, R.G. and Edwardson, J.R. (1974) Transmission of a bacilliform virus of sowthistle and *Bidens pilosa*. *Phytopathology*, **64**, 840–845.

Corbett, A.H. and Silver, P.A. (1997) Nucleocytoplasmic transport of macromolecules. *Microbiol. Mol. Biol. Rev.* **61**, 193–211.

Cronshaw, J.M., Krutchinsky, A.N., Zhang, W., Chait, B.T. and Matunis, M.J. (2002) Proteomic analysis of the mammalian nuclear pore complex. *J. Cell Biol.* **158**, 915–927.

Cros, J.F. and Palese, P. (2003) Trafficking of viral genomic RNA into and out of the nucleus: influenza, Thogoto and Borna disease viruses. *Virus Res.* **95**, 3–12.

Cullen, B.R. (2001) Journey to the center of the cell. *Cell*, **105**, 697–700.

Czosneck, H. (1999) *Tomato yellow leaf curl virus-Israel.* CMI/AAB Description of Plant Viruses no. 368. URL: http://www.dpvweb.net/dpv/show-dpv.php?dpvno=368

Dabauvalle, M.C., Schulz, B., Scheer, U. and Peters, R. (1988) Inhibition of nuclear accumulation of karyophilic proteins in living cells by microinjection of the lectin wheat germ agglutinin. *Exp. Cell Res.* **174**, 291–296.

Das, T., Pattnaik, A.K., Takacs, A.M., Li, T., Hwang, L.N. and Banerjee, A.K. (1997) Basic amino acid residues at the carboxy-terminal eleven amino acid region of the phosphoprotein (P) are required for transcription but

not for replication of vesicular stomatitis virus genome RNA. *Virology*, **238**, 103–114.

Davis, L.I. (1995) The nuclear pore complex. *Annu. Rev. Biochem.* **64**, 865–896.

Deane, R., Schafer, W., Zimmermann, H.P., Mueller, L., Görlich, D., Prehn, S., Ponstingl, H. and Bischoff, F.R. (1997) Ran-binding protein 5 (RanBP5) is related to the nuclear transport factor importin-beta but interacts differently with RanBP1. *Mol. Cell. Biol.* **17**, 5087–5096.

Dickinson, V.J., Halder, J. and Woolston, C.J. (1996) The product of maize streak virus ORF V1 is associated with secondary plasmodesmata and is first detected with the onset of viral lesions. *Virology*, **220**, 51–59.

Dingwall, C. (1991) Transport across the nuclear envelope: enigmas and explanations. *Bioessays*, **13**, 213–218.

Dingwall, C. and Laskey, R.A. (1991) Nuclear targeting sequences — a consensus? *Trends Biochem. Sci.* **16**, 478–481.

Dong, X., van Wezel, R., Stanley, J. and Hong, Y. (2003) Functional characterization of the nuclear localization signal for a suppressor of posttranscriptional gene silencing. *J. Virol.* **77**, 7026–7033.

Dworetzky, S.I. and Feldherr, C.M. (1988) Translocation of RNA-coated gold particles through the nuclear pores of oocytes. *J. Cell Biol.* **106**, 575–584.

Efthymiadis, A., Shao, H., Hubner, S. and Jans, D.A. (1997) Kinetic characterization of the human retinoblastoma protein bipartite nuclear localization sequence (NLS) *in vivo* and *in vitro*. A comparison with the SV40 large T-antigen NLS. *J. Biol. Chem.* **272**, 22134–22139.

Elmer, J.S., Brand, L., Sunter, G., Gardiner, W.E., Bisaro, D.M. and Rogers, S.G. (1988) Genetic analysis of the tomato golden mosaic virus. II. Requirement for the product of the highly conserved ALI coding sequence for replication. *Nucleic Acids Res.* **16**, 7043–7060.

Enenkel, C., Blobel, G. and Rexach, M. (1995) Identification of a yeast karyopherin heterodimer that targets import substrate to mammalian nuclear pore complexes. *J. Biol. Chem.* **270**, 16499–16502.

Erkmann, J.A. and Kutay, U. (2004) Nuclear export of mRNA: from the site of transcription to the cytoplasm. *Exp. Cell Res.* **296**, 12–20.

Feldherr, C.M., Kallenbach, E. and Schultz, N. (1984) Movement of a karyophilic protein through the nuclear pores of oocytes. *J. Cell Biol.* **99**, 2216–2222.

Fischer, U., Huber, J., Boelens, W.C., Mattaj, I.W. and Luhrmann, R. (1995) The HIV-1 Rev. activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. *Cell*, **82**, 475–483.

Fischer, U., Meyer, S., Teufel, M., Heckel, C., Luhrmann, R. and Rautmann, G. (1994) Evidence that HIV-1 Rev. directly promotes the nuclear export of unspliced RNA. *EMBO J.* **13**, 4105–4112.

Fornerod, M., Ohno, M., Yoshida, M. and Mattaj, I.W. (1997) CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell*, **90**, 1051–1060.

Frank, A., Guilley, H., Jonard, G., Richards, K. and Hirth, L. (1980) Nucleotide sequence of cauliflower mosaic virus DNA. *Cell*, **21**, 285–294.

Fridell, R.A., Fischer, U., Luhrmann, R., Meyer, B.E., Meinkoth, J.L., Malim, M.H. and Cullen, B.R. (1996) Amphibian transcription factor IIIA proteins contain a sequence element functionally equivalent to the nuclear export signal of human immunodeficiency virus type 1 Rev. *Proc. Natl Acad. Sci. USA*, **93**, 2936–2940.

Fridell, R.A., Truant, R., Thorne, L., Benson, R.E. and Cullen, B.R. (1997) Nuclear import of hnRNP A1 is mediated by a novel cellular cofactor related to karyopherin-beta. *J. Cell Sci.* **110**, 1325–1331.

Fried, H. and Kutay, U. (2003) Nucleocytoplasmic transport: taking an inventory. *Cell. Mol. Life Sci.* **60**, 1659–1688.

Friedler, A., Zakai, N., Karni, O., Broder, Y.C., Baraz, L., Kotler, M., Loyter, A. and Gilon, C. (1998) Backbone cyclic peptide, which mimics the nuclear localization signal of human immunodeficiency virus type 1 matrix protein, inhibits nuclear import and virus production in nondividing cells. *Biochemistry*, **37**, 5616–5622.

Gafni, Y. (2003) *Tomato yellow leaf curl virus*, the intracellular dynamics of a plant DNA virus. *Mol. Plant Pathol.* **4**, 9–15.

Gao, Y., Greenfield, N.J., Cleverley, D.Z. and Lenard, J. (1996) The transcriptional form of the phosphoprotein of vesicular stomatitis virus is a trimer: structure and stability. *Biochemistry*, **35**, 14569–14573.

Garcia-Bustos, J., Heitman, J. and Hall, M.N. (1991) Nuclear protein localization. *Biochim. Biophys. Acta*, **1071**, 83–101.

Goldfarb, D.S., Corbett, A.H., Mason, D.A., Harreman, M.T. and Adam, S.A. (2004) Importin alpha: a multipurpose nuclear-transport receptor. *Trends Cell Biol.* **14**, 505–514.

Goodin, M.M., Austin, J., Tobias, R., Fujita, M., Morales, C. and Jackson, A.O. (2001) Interactions and nuclear import of the N and P proteins of sonchus yellow net virus, a plant nucleorhabdovirus. *J. Virol.* **75**, 9393–9406.

Goodin, M.M., Dietzgen, R.G., Schichnes, D., Ruzin, S. and Jackson, A.O. (2002) pGD vectors: versatile tools for the expression of green and red fluorescent protein fusions in agroinfiltrated plant leaves. *Plant J.* **31**, 375–383.

Görlich, D. (1997) Nuclear protein import. *Curr. Opin. Cell Biol.* **9**, 412–419.

Görlich, D., Kostka, S., Kraft, R., Dingwall, C., Laskey, R.A., Hartmann, E. and Prehn, S. (1995a) Two different subunits of importin cooperate to recognize nuclear localization signals and bind them to the nuclear envelope. *Curr. Biol.* **5**, 383–392.

Görlich, D. and Kutay, U. (1999) Transport between the cell nucleus and the cytoplasm. *Annu. Rev. Cell Dev. Biol.* **15**, 607–660.

Görlich, D., Vogel, F., Mills, A.D., Hartmann, E. and Laskey, R.A. (1995b) Distinct functions for the two importin subunits in nuclear protein import. *Nature*, **377**, 246–248.

Guerra-Peraza, O., de Tapia, M., Hohn, T. and Hemmings-Mieszczak, M. (2000) Interaction of the cauliflower mosaic virus coat protein with the pregenomic RNA leader. *J. Virol.* **74**, 2067–2072.

Haizel, T., Merkle, T., Pay, A., Fejes, E. and Nagy, F. (1997) Characterization of proteins that interact with the GTP-bound form of the regulatory GTPase Ran in Arabidopsis. *Plant J.* **11**, 93–103.

Hall, M.N., Hereford, L. and Herskowitz, I. (1984) Targeting of *E. coli* beta-galactosidase to the nucleus in yeast. *Cell*, **36**, 1057–1065.

Hanley-Bowdoin, L., Elmer, J.S. and Rogers, S.G. (1990) Expression of functional replication protein from tomato golden mosaic virus in transgenic tobacco plants. *Proc. Natl Acad. Sci. USA*, **87**, 1446–1450.

Harel, A. and Forbes, D.J. (2004) Importin beta: conducting a much larger cellular symphony. *Mol. Cell*, **16**, 319–330.

Hartitz, M.D., Sunter, G. and Bisaro, D.M. (1999) The tomato golden mosaic virus transactivator (TrAP) is a single-stranded DNA and zinc-binding phosphoprotein with an acidic activation domain. *Virology*, **263**, 1–14.

Heese-Peck, A., Cole, R.N., Borkhsenious, O.N., Hart, G.W. and Raikhel, N.V. (1995) Nuclear pore complex proteins from higher plants are modified by novel O-linked oligosaccharides. *Plant Cell*, **7**, 1459–1471.

Hicks, G.R. and Raikhel, N.V. (1995) Protein import into the nucleus: an integrated view. *Annu. Rev. Cell Dev. Biol.* **11**, 155–188.

Hicks, G.R., Smith, H.M.S., Lobreaux, S. and Raikhel, N.V. (1996) Nuclear import in permeabilized protoplasts from higher plants has unique features. *Plant Cell*, **8**, 1337–1352.

Hirsch, V.M., Dapolito, G., Goeken, R. and Campbell, B.J. (1995) Phylogeny and natural history of the primate lentiviruses, SIV and HIV. *Curr. Opin. Genet. Dev.* **5**, 798–806.

Hu, C.D., Chinenov, Y. and Kerppola, T.K. (2002) Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. *Mol. Cell*, **9**, 789–798.

Hu, W. and Jans, D.A. (1999) Efficiency of importin alpha/beta-mediated nuclear localization sequence recognition and nuclear import. *J. Biol. Chem.* **274**, 15820–15827.

Hu, C.D. and Kerppola, T.K. (2003) Simultaneous visualization of multiple protein interactions in living cells using multicolor fluorescence complementation analysis. *Nat. Biotechnol.* **21**, 539–545.

Hubner, S., Smith, H.M.S., Hu, W., Chan, C.K., Rihs, H.P., Paschal, B.M., Raikhel, N.V. and Jans, D.A. (1999) Plant importin alpha binds nuclear localization sequences with high affinity and can mediate nuclear import independent of importin beta. *J. Biol. Chem.* **274**, 22610–22617.

Hubner, S., Xiao, C.Y. and Jans, D.A. (1997) The protein kinase CK2 site (Ser111/112) enhances recognition of the simian virus 40 large T-antigen nuclear localization sequence by importin. *J. Biol. Chem.* **272**, 17191–17195.

Hull, R. and Covey, S.N. (1985) Cauliflower mosaic virus: pathways of infection. *Bioessays*, **3**, 160–163.

Hwang, L.N., Englund, N., Das, T., Banerjee, A.K. and Pattnaik, A.K. (1999) Optimal replication activity of vesicular stomatitis virus RNA polymerase requires phosphorylation of a residue(s) at carboxy-terminal domain II of its accessory subunit, phosphoprotein P. *J. Virol.* **73**, 5613–5620.

Izaurralde, E., Kann, M., Pante, N., Sodeik, B. and Hohn, T. (1999) Viruses, microorganisms and scientists meet the nuclear pore. *EMBO J.* **18**, 289–296.

Jackson, A.O. (1978) Partial characterization of the structural proteins of sonchus yellow net virus. *Virology*, **87**, 172–181.

Jackson, A.O. (1987) Biology, structure, and replication of plant rhabdoviruses. In *The Rhabdoviruses* (Wagner, R.R., ed.), pp. 427–508. New York: Plenum Press.

Jackson, A.O. and Christie, S.R. (1979) *Sonchus yellow net virus.* CMI/AAB Description of Plant Viruses no. 205. URL: http://www.dpvweb.net/dpv/showdpv.php?dpvno=205

Jackson, A.O., Dietzgen, R.G., Goodin, M.M., Bragg, J.N. and Deng, M. (2005) Biology of plant rhabdoviruses. *Annu. Rev. Phytopathol.* **43**, 623–660.

Jäkel, S., Albig, W., Kutay, U., Bischoff, F.R., Schwamborn, K., Doenecke, D. and Görlich, D. (1999) The importin beta/importin 7 heterodimer is a functional nuclear import receptor for histone H1. *EMBO J.* **18**, 2411–2423.

Jiang, C.J., Imamoto, N., Matsuki, R., Yoneda, Y. and Y. (1998a) *In vitro* characterization of rice importin beta1: molecular interaction with nuclear transport factors and mediation of nuclear protein import. *FEBS Lett.* **437**, 127–130.

Jiang, C.J., Imamoto, N., Matsuki, R., Yoneda, Y. and Yamamoto, N. (1998b) Functional characterization of a plant importin alpha homologue. Nuclear localization signal (NLS)-selective binding and mediation of nuclear import of NLS proteins *in vitro*. *J. Biol. Chem.* **273**, 24083–24087.

Jupin, I., De Kouchkovsky, F., Jouanneau, F. and Gronenborn, B. (1994) Movement of tomato yellow leaf curl geminivirus (TYLCV): involvement of the protein encoded by ORF C4. *Virology*, **204**, 82–90.

Kalderon, D., Richardson, W.D., Markham, A.F. and Smith, A.E. (1984a) Sequence requirements for nuclear location of simian virus 40 large-T antigen. *Nature*, **311**, 33–38.

Kalderon, D., Roberts, B.L., Richardson, W.D. and Smith, A.E. (1984b) A short amino acid sequence able to specify nuclear location. *Cell*, **39**, 499–509.

Kann, M., Bischof, A. and Gerlich, W.H. (1997) *In vitro* model for the nuclear transport of the hepadnavirus genome. *J. Virol.* **71**, 1310–1316.

Kann, M., Sodeik, B., Vlachou, A., Gerlich, W.H. and Helenius, A. (1999) Phosphorylation-dependent binding of hepatitis B virus core particles to the nuclear pore complex. *J. Cell Biol.* **145**, 45–55.

Karni, O., Friedler, A., Zakai, N., Gilon, C. and Loyter, A. (1998) A peptide derived from the N-terminal region of HIV-1 Vpr promotes nuclear import in permeabilized cells: elucidation of the NLS region of the Vpr. *FEBS Lett.* **429**, 421–425.

Karsies, A., Hohn, T. and Leclerc, D. (2001) Degradation signals within both terminal domains of the cauliflower mosaic virus capsid protein precursor. *Plant J.* **27**, 335–343.

Karsies, A., Merkle, T., Szurek, B., Bonas, U., Hohn, T. and Leclerc, D. (2002) Regulated nuclear targeting of cauliflower mosaic virus. *J. Gen. Virol.* **83**, 1783–1790.

Komeili, A. and O'Shea, E.K. (2001) New perspectives on nuclear transport. *Annu. Rev. Genet.* **35**, 341–364.

Kotera, I., Sekimoto, T., Miyamoto, Y., Saiwaki, T., Nagoshi, E., Sakagami, H., Kondo, H. and Yoneda, Y. (2005) Importin alpha transports CaMKIV to the nucleus without utilizing importin beta. *EMBO J.* **24**, 942–951.

Kotlizky, G., Boulton, M.I., Pitaksutheepong, C., Davies, J.W. and Epel, B.L. (2000) Intracellular and intercellular movement of maize streak geminivirus V1 and V2 proteins transiently expressed as green fluorescent protein fusions. *Virology*, **274**, 32–38.

Kraemer, D.M., Strambio-de-Castillia, C., Blobel, G. and Rout, M.P. (1995) The essential yeast nucleoporin NUP159 is located on the cytoplasmic side of the nuclear pore complex and serves in karyopherin-mediated binding of transport substrate. *J. Biol. Chem.* **270**, 19017–19021.

Krake, L.R., Rezaian, M.A. and Dry, I.B. (1998) Expression of the tomato leaf curl geminivirus *C4* gene produces viruslike symptoms in transgenic plants. *Mol. Plant–Microbe Interact.* **11**, 413–417.

Krichevsky, A., Graessmann, A., Nissim, A., Piller, S.C., Zakai, N. and Loyter, A. (2003) Antibody fragments selected by phage display against the nuclear localization signal of the HIV-1 Vpr protein inhibit nuclear import in permeabilized and intact cultured cells. *Virology*, **305**, 77–92.

Krichevsky, A., Rusnati, M., Bugatti, A., Waigmann, E., Shohat, S. and Loyter, A. (2005) The fd phage and a peptide derived from its p8 coat protein interact with the HIV-1 Tat-NLS and inhibit its biological functions. *Antiviral Res.* **66**, 67–78.

Kunik, T., Palanichelvam, K., Czosnek, H., Citovsky, V. and Gafni, Y. (1998) Nuclear import of the capsid protein of tomato yellow leaf curl virus (TYLCV) in plant and insect cells. *Plant J.* **13**, 393–399.

Kutay, U., Bischoff, F.R., Kostka, S., Kraft, R. and Görlich, D. (1997) Export of importin alpha from the nucleus is mediated by a specific nuclear transport factor. *Cell*, **90**, 1061–1071.

Kutay, U. and Guttinger, S. (2005) Leucine-rich nuclear-export signals: born to be weak. *Trends Cell Biol.* **15**, 121–124.

Lacroix, B., Vaidya, M., Tzfira, T. and Citovsky, V. (2005) The VirE3 protein of *Agrobacterium* mimics a host cell function required for plant genetic transformation. *EMBO J.* **24**, 428–437.

Lazarowitz, S.G. (1992) Geminiviruses: genome structure and gene function. *Crit. Rev. Plant Sci.* **11**, 327–349.

Lazarowitz, S.G. and Beachy, R.N. (1999) Viral movement proteins as probes for intracellular and intercellular trafficking in plants. *Plant Cell*, **11**, 535–548.

Lazarowitz, S.G., Pinder, A.J., Damsteegt, V.D. and Rogers, S.G. (1989) Maize streak virus genes essential for systemic spread and symptom development. *EMBO J.* **8**, 1023–1032.

Leclerc, D., Chapdelaine, Y. and Hohn, T. (1999) Nuclear targeting of the cauliflower mosaic virus coat protein. *J. Virol.* **73**, 553–560.

Leuzinger, H., Ziegler, U., Schraner, E.M., Fraefel, C., Glauser, D.L., Heid, I., Ackermann, M., Mueller, M. and Wild, P. (2005) Herpes simplex virus 1 envelopment follows two diverse pathways. *J. Virol.* **79**, 13047–13059.

Li, X.H. and Carrington, J.C. (1993) Nuclear transport of tobacco etch potyviral RNA-dependent RNA polymerase is highly sensitive to sequence alterations. *Virology*, **193**, 951–958.

Li, J., Krichevsky, A., Vaidya, M., Tzfira, T. and Citovsky, V. (2005) Uncoupling of the functions of the *Arabidopsis* VIP1 protein in transient and stable plant genetic transformation by *Agrobacterium*. *Proc. Natl Acad. Sci. USA*, **102**, 5733–5738.

Li, X.H., Valdez, P., Olvera, R.E. and Carrington, J.C. (1997) Functions of the tobacco etch virus RNA polymerase (NIb): subcellular transport and protein–protein interaction with VPg/proteinase (NIa). *J. Virol.* **71**, 1598–1607.

Liu, H., Boulton, M.I. and Davies, J.W. (1997) Maize streak virus coat protein binds single- and double-stranded DNA *in vitro*. *J. Gen. Virol.* **78**, 1265–1270.

Liu, H., Boulton, M.I., Oparka, K.J. and Davies, J.W. (2001) Interaction of the movement and coat proteins of *Maize streak virus*: implications for the transport of viral DNA. *J. Gen. Virol.* **82**, 35–44.

Liu, H., Boulton, M.I., Thomas, C.L., Prior, D.A., Oparka, K.J. and Davies, J.W. (1999) Maize streak virus coat protein is karyophyllic and facilitates nuclear transport of viral DNA. *Mol. Plant–Microbe Interact.* **12**, 894–900.

Lucy, A.P., Guo, H.S., Li, W.X. and Ding, S.W. (2000) Suppression of post-transcriptional gene silencing by a plant viral protein localized in the nucleus. *EMBO J.* **19**, 1672–1680.

Lyman, S.K., Guan, T., Bednenko, J., Wodrich, H. and Gerace, L. (2002) Influence of cargo size on Ran and energy requirements for nuclear protein import. *J. Cell Biol.* **159**, 55–67.

Martins, C.R.F., Johnson, J.A., Lawrence, D.M., Choi, T.J., Pisi, A., Tobin, S.L., Lapidus, D., Wagner, J.D.O., Ruzin, S., McDonald, K. and Jackson, A.O. (1998) Sonchus yellow net rhabdovirus nuclear viroplasms contain polymerase-associated proteins. *J. Virol.* **72**, 5669–5679.

Masters, P.S. and Banerjee, A.K. (1988) Resolution of multiple complexes of phosphoprotein NS with nucleocapsid protein N of vesicular stomatitis virus. *J. Virol.* **62**, 2651–2657.

Matsuki, R., Iwasaki, T., Shoji, K., Jiang, C.J. and Yamamoto, N. (1998) Isolation and characterization of two importin-beta genes from rice. *Plant Cell Physiol.* **39**, 879–884.

Mayers, C.N., Palukaitis, P. and Carr, J.P. (2000) Subcellular distribution analysis of the cucumber mosaic virus 2b protein. *J. Gen. Virol.* **81**, 219–226.

McGarry, R.C., Barron, Y.D., Carvalho, M.F., Hill, J.E., Gold, D., Cheung, E., Kraus, W.L. and Lazarowitz, S.G. (2003) A novel *Arabidopsis* acetyltransferase interacts with the geminivirus movement protein NSP. *Plant Cell*, **15**, 1605–1618.

Meier, I. (2005) Nucleocytoplasmic trafficking in plant cells. *Int. Rev. Cytol.* **244**, 95–135.

Ménissier, J., de Murcia, G., Lebeurier, G. and Hirth, L. (1983) Electron microscopic studies of the different topological forms of the cauliflower mosaic virus DNA: knotted encapsidated DNA and nuclear minichromosome. *EMBO J.* **2**, 1067–1071.

Merkle, T., Haizel, T., Matsumoto, T., Harter, K., Dallmann, G. and Nagy, F. (1994) Phenotype of the fission yeast cell cycle regulatory mutant *pim1–46* is suppressed by a tobacco cDNA encoding a small, Ran-like GTP-binding protein. *Plant J.* **6**, 555–565.

Merkle, T., Lecrec, D., Marshallsay, C. and Nagy, F. (1996) A plant *in vitro* system for the nuclear import of proteins. *Plant J.* **10**, 1177–1186.

Mlotshwa, S., Verver, J., Sithole-Niang, I., Gopinath, K., Carette, J., van Kammen, A. and Wellink, J. (2002) Subcellular location of the helper component-proteinase of Cowpea aphid-borne mosaic virus. *Virus Genes*, **25**, 207–216.

Moriones, E. and Navas-Castillo, J. (2000) Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus Res.* **71**, 123–134.

Morris-Krsinich, B.A.M., Mullineaux, P.M., Donson, J., Boulton, M.I., Markham, P.G., Short, M.N. and Davies, J.W. (1985) Bidirectional transcription of maize streak virus DNA and identification of the coat protein gene. *Nucleic Acids Res.* **13**, 7237–7256.

Mosammaparast, N. and Pemberton, L.F. (2004) Karyopherins: from nuclear-transport mediators to nuclear-function regulators. *Trends Cell Biol.* **14**, 547–556.

Mullineaux, P.M., Boulton, M.I., Bowyer, P., van der Vlugt, R., Marks, M., Donson, J. and Davies, J.W. (1988) Detection of a non-structural protein of MW11000 encoded by the virion DNA of maize streak virus. *Plant Mol. Biol.* **11**, 57–66.

Nagar, S., Pedersen, T.J., Carrick, K.M., Hanley-Bowdoin, L. and Robertson, D. (1995) A geminivirus induces expression of a host DNA synthesis protein in terminally differentiated plant cells. *Plant Cell*, **7**, 705–719.

Nair, R., Carter, P. and Rost, B. (2003) NLSdb: database of nuclear localization signals. *Nucleic Acids Res.* **31**, 397–399.

Nakhla, M.K. and Maxwell, D.P. (1997) Epidemiology and management of tomato yellow leaf curl disease. *Plant Virus Dis. Control.* **43**, 565–583.

Navot, N., Pichersky, E., Zeidan, M., Zamir, D. and Czosnek, H. (1991) Tomato yellow leaf curl virus: a whitefly transmitted geminivirus with a single genomic component. *Virology*, **185**, 151–161.

Newbold, J.E., Xin, H., Tencza, M., Sherman, G., Dean, J., Bowden, S. and Locarnini, S. (1995) The covalently closed duplex form of the hepadnavirus genome exists in situ as a heterogeneous population of viral minichromosomes. *J. Virol.* **69**, 3350–3357.

Nigg, E.A. (1997) Nucleocytoplasmic transport: signals, mechanisms and regulation. *Nature*, **386**, 779–787.

Noueiry, A.O., Lucas, W.J. and Gilbertson, R.L. (1994) Two proteins of a plant DNA virus coordinate nuclear and plasmodesmal transport. *Cell*, **76**, 925–932.

Ojala, P.M., Sodeik, B., Ebersold, M.W., Kutay, U. and Helenius, A. (2000) Herpes simplex virus type 1 entry into host cells: reconstitution of capsid binding and uncoating at the nuclear pore complex *in vitro*. *Mol. Cell. Biol.* **20**, 4922–4931.

Olszewski, N.E. and Guilfoyle, T.J. (1983) Nuclei purified from cauliflower mosaic virus-infected turnip leaves contain subgenomic, covalently closed circular cauliflower mosaic virus DNAs. *Nucleic Acids Res.* **11**, 8901–8914.

Olszewski, N.E., Hagen, G. and Guilfoyle, T.J. (1982) A transcriptionally active, covalently closed minichromosome of cauliflower mosaic virus DNA isolated from turnip leaves. *Cell*, **29**, 395–402.

Ossareh-Nazari, B., Gwizdek, C. and Dargemont, C. (2001) Protein export from the nucleus. *Traffic*, **2**, 684–689.

Palukaitis, P. and Garcia-Arenal, F. (2003) *Cucumber mosaic virus.* CMI/AAB Description of Plant Viruses no. 400. URL: http://www.dpvweb.net/dpv/showdpv.php?dpvno=400

Panté, N. and Kann, M. (2002) Nuclear pore complex is able to transport macromolecules with diameters of about 39 nm. *Mol. Biol. Cell*, **13**, 425–434.

Pascal, E., Goodlove, P.E., Wu, L.C. and Lazarowitz, S.G. (1993) Transgenic tobacco plants expressing the geminivirus BL1 protein exhibit symptoms of viral disease. *Plant Cell*, **5**, 795–807.

Pascal, E., Sanderfoot, A.A., Ward, B.M., Medville, R., Turgeon, R. and Lazarowitz, S.G. (1994) The geminivirus BR1 movement protein binds single-stranded DNA and localizes to the cell nucleus. *Plant Cell*, **6**, 995–1006.

Pfeiffer, P. and Hohn, T. (1983) Involvement of reverse transcription in the replication of cauliflower mosaic virus: a detailed model and test of some aspects. *Cell*, **33**, 781–789.

Pilartz, M. and Jeske, H. (1992) Abutilon mosaic geminivirus double-stranded DNA is packed into minichromosomes. *Virology*, **189**, 800–802.

Pollard, V.W., Michael, W.M., Nakielny, S., Siomi, M.C., Wang, F. and Dreyfuss, G. (1996) A novel receptor-mediated nuclear protein import pathway. *Cell*, **86**, 985–994.

Powers, M.A. and Forbes, D.J. (1994) Cytosolic factors in nuclear import: what's importin? *Cell*, **79**, 931–934.

Pringle, C.R. (1999) Virus nomenclature. *Arch. Virol.* **144**, 1463–1466.

Qin, S., Ward, B.M. and Lazarowitz, S.G. (1998) The bipartite geminivirus coat protein aids BR1 function in viral movement by affecting the accumulation of viral single-stranded DNA. *J. Virol.* **72**, 9247–9256.

Radu, A., Moore, M.S. and Blobel, G. (1995) The peptide repeat domain of nucleoporin Nup98 functions as a docking site in transport across the nuclear pore complex. *Cell*, **81**, 215–222.

Reichelt, R., Holzenburg, A., Buhle,E.L., JrJarnik, M., Engel, A. and Aebi, U. (1990) Correlation between structure and mass distribution of the nuclear pore complex and of distinct pore complex components. *J. Cell Biol.* **110**, 883–894.

Rexach, M. and Blobel, G. (1995) Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. *Cell*, **83**, 683–692.

Rhee, Y., Gurel, F., Gafni, Y., Dingwall, C. and Citovsky, V. (2000) A genetic system for detection of protein nuclear import and export. *Nat. Biotechnol.* **18**, 433–437.

Rigden, J.E., Krake, L.R., Rezaian, M.A. and Dry, I.B. (1994) ORF C4 of tomato leaf curl geminivirus is a determinant of symptom severity. *Virology*, **204**, 847–850.

Robbins, J., Dilworth, S.M., Laskey, R.A. and Dingwall, C. (1991) Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. *Cell*, **64**, 615–623.

Roe, T., Reynolds, T.C., Yu, G. and Brown, P.O. (1993) Integration of murine leukemia virus DNA depends on mitosis. *EMBO J.* **12**, 2099–2108.

Roizman, B. and Sears, A.E. (1996) Herpes simplex viruses and their replication. In *Virology*, 3rd edn. (Fields, B.N., Knipe, D.M. and Howely, P.M., eds). Philadelphia: Lippincott-Raven, pp. 2231–2295.

Rojas, M.R., Hagen, C., Lucas, W.J. and Gilbertson, R.L. (2005) Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. *Annu. Rev. Phytopathol.* **43**, 361–394.

Rojas, M.R., Jiang, H., Salati, R., Xoconostle-Cázares, B., Sudarshana, M.R., Lucas, W.J. and Gilbertson, R.L. (2001) Functional analysis of proteins involved in movement of the monopartite begomovirus, *Tomato yellow leaf curl virus. Virology*, **291**, 110–125.

Rojas, M.R., Noueiry, A.O., Lucas, W.J. and Gilbertson, R.L. (1998) Bean dwarf mosaic geminivirus movement proteins recognize DNA in a form- and size-specific manner. *Cell*, **95**, 105–113.

Rose, J.K. and Whitt, M.A. (2001) *Rhabdoviridae*: the viruses and their replication. In *Field's Virology*, Vol. 1, 4th edn (Knipe, D.M. and Howely, P.M., eds), pp. 1221–1244. Philadelphia: Lippincott, Williams & Wilkins.

Rothnie, H.M., Chapdelaine, Y. and Hohn, T. (1994) Pararetroviruses and retroviruses: a comparative review of viral structure and gene expression strategies. *Adv. Virus. Res.* **44**, 1–67.

Rout, M.P., Aitchison, J.D., Suprapto, A., Hjertaas, K., Zhao, Y. and Chait, B.T. (2000) The yeast nuclear pore complex: composition, architecture, and transport mechanism. *J. Cell Biol.* **148**, 635–651.

Rout, M.P. and Blobel, G. (1993) Isolation of the yeast nuclear pore complex. *J. Cell Biol.* **123**, 771–783.

Ryabov, E.V., Kim, S.H. and Taliansky, M. (2004) Identification of a nuclear localization signal and nuclear export signal of the umbraviral long-distance RNA movement protein. *J. Gen. Virol.* **85**, 1329–1333.

Ryabov, E.V., Oparka, K.J., Santa Cruz, S., Robinson, D.J. and Taliansky, M.E. (1998) Intracellular location of two groundnut rosette umbravirus proteins delivered by PVX and TMV vectors. *Virology*, **242**, 303–313.

Ryabova, L.A., Pooggin, M.M. and Hohn, T. (2002) Viral strategies of translation initiation: ribosomal shunt and reinitiation. *Prog. Nucleic Acids Res. Mol. Biol.* **72**, 1–39.

Rybicki, E.P., Briddon, R.W., Brown, J.K., Fauquet, C.M., Maxwell, D.P., Stanley, J., Harrison, B.D., Markham, P.G., Bisaro, D.M. and Robinson, D. (2000) Family *Geminiviridae*. In *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses* (Van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E., Estes, M., Lemon, S., Maniloff, J., Mayo, M.A., McGeoch, D., Pringle, C. and Wickner, R., eds), pp. 285–297. San Diego: Academic Press.

Sanderfoot, A.A., Ingham, D.J. and Lazarowitz, S.G. (1996) A viral movement protein as a nuclear shuttle. *Plant Physiol.* **110**, 23–33.

Sanderfoot, A.A. and Lazarowitz, S.G. (1995) Cooperation in viral movement: the geminivirus BL1 movement protein interacts with BR1 and redirects it from the nucleus to the cell periphery. *Plant Cell*, **7**, 1185–1194.

Sanderfoot, A.A. and Lazarowitz, S.G. (1996) Getting it together in plant virus movement: cooperative interactions between bipartite geminivirus movement proteins. *Trends Cell Biol.* **6**, 353–358.

Schaad, M.C., Haldeman-Cahill, R., Cronin, S. and Carrington, J.C. (1996) Analysis of the VPg-proteinase (NIa) encoded by tobacco etch potyvirus: effects of mutations on subcellular transport, proteolytic processing, and genome amplification. *J. Virol.* **70**, 7039–7048.

Schlenstedt, G., Smirnova, E., Deane, R., Solsbacher, J., Kutay, U., Görlich, D., Ponstingl, H. and Bischoff, F.R. (1997) Yrb4p, a yeast Ran-GTP-binding protein involved in import of ribosomal protein L25 into the nucleus. *EMBO J.* **16**, 6237–6249.

Scofield, G.N., Beven, A.F., Shaw, P.J. and Doonan, J.H. (1992) Identification and localization of a nucleoporin-like protein component of the plant nuclear matrix. *Planta*, **187**, 414–420.

Shepherd, R.J. (1981) *Cauliflower mosaic virus.* CMI/AAB Description of Plant Viruses no. 243. URL: http://www.dpvweb.net/dpv/showdpv.php?dpvno=243

Shepherd, R.J. (1989) Biochemistry of DNA plant viruses. In *The Biochemistry of Plants*, Vol. 15 (Marcus, A., ed.), pp. 563–616 New York: Academic Press.

Smith, A.E. and Helenius, A. (2004) How viruses enter animal cells. *Science*, **304**, 237–242.

Smith, H.M.S., Hicks, G.R. and Raikhel, N.V. (1997) Importin alpha from *Arabidopsis thaliana* is a nuclear import receptor that recognizes three classes of import signals. *Plant Physiol.* **114**, 411–417.

Smith, H.M.S. and Raikhel, N.V. (1999) Protein targeting to the nuclear pore. What can we learn from plants? *Plant Physiol.* **119**, 1157–1164.

Spear, P.G. (1993) Entry of alphaherpesviruses into cells. *Semin. Virol.* **4**, 167–180.

Stade, K., Ford, C.S., Guthrie, C. and Weis, K. (1997) Exportin 1 (Crm1p) is an essential nuclear export factor. *Cell*, **90**, 1041–1050.

Stanley, J., Boulton, M.I. and Davies, J.W. (1999) *Geminiviridae. Encyclopedia of Life Sciences (www.els.net)*. London: Nature Publishing Group.

Sunter, G., Hertitz, M.D., Hormuzdi, S.G., Brough, C.L. and Bisaro, D.M. (1990) Genetic analysis of tomato golden mosaic virus: ORF AL2 is required for coat protein accumulation while ORFAL3 is necessary for efficient DNA replication. *Virology*, **179**, 69–77.

Takacs, A.M. and Banerjee, A.K. (1995) Efficient interaction of the vesicular stomatitis P protein with the L or N protein in cells expressing recombinant proteins. *Virology*, **208**, 821–826.

Takacs, A.M., Das, T. and Banerjee, A.K. (1993) Mapping of interacting domains between the nucleocapsid protein and the phosphoprotein of vesicular stomatitis virus by using a two-hybrid system. *Proc. Natl Acad. Sci. USA*, **90**, 10375–10379.

Timmermans, M., Das, O. and Messing, J. (1994) Geminiviruses and their uses as extrachromosomal replicons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 79–112.

Tordo, N., Benmansour, A., Calisher, C., Dietzgen, R.G., Fang, R.X., Jackson, A.O., Kurath, G., Nadin-Davis, S., Tesh, R.B. and Walker, P.J. (2005) Family *Rhabdoviridae*. In *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses* (Fauquet, C.M., Mayo, M.A., Maniloff, J., Dessenberger, U. and Ball, L.A., eds), pp. 623–644. San Diego: Elsevier/Academic Press.

Torruella, M., Gordon, K. and Hohn, T. (1989) Cauliflower mosaic virus produces an aspartic proteinase to cleave its polyproteins. *EMBO J.* **8**, 2819–2825.

Trinks, D., Rajeswaran, R., Shivaprasad, P.V., Akbergenov, R., Oakeley, E.J., Veluthambi, K., Hohn, T. and Pooggin, M.M. (2005) Suppression of RNA silencing by a geminivirus nuclear protein, AC2, correlates with transactivation of host genes. *J. Virol.* **79**, 2517–2527.

Truant, R. and Cullen, B.R. (1999) The arginine-rich domains present in HIV-1 Tat and Rev. function as direct importin beta dependent nuclear localization signals. *Mol. Cell. Biol.* **19**, 1210–1217.

Tsai, C.W., Redinbaugh, M.G., Willie, K.J., Reed, S., Goodin, M. and Hogenhout, S.A. (2005) Complete genome sequence and in planta subcellular localization of maize fine streak virus proteins. *J. Virol.* **79**, 5304–5314.

Tuttleman, J.S., Pourcel, C. and Summers, J. (1986) Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. *Cell*, **47**, 451–460.

Tzfira, T., Vaidya, M. and Citovsky, V. (2004) Involvement of targeted proteolysis in plant genetic transformation by *Agrobacterium*. *Nature*, **431**, 87–92.

Uhrig, J.F., Canto, T., Marshall, D. and MacFarlane, S.A. (2004) Relocalization of nuclear ALY proteins to the cytoplasm by the tomato bushy stunt virus P19 pathogenicity protein. *Plant Physiol.* **135**, 2411–2423.

Ullman, K.S., Powers, M.A. and Forbes, D.J. (1997) Nuclear export receptors: from importin to exportin. *Cell*, **90**, 967–970.

Van Wezel, R., Liu, H., Wu, Z., Stanley, J. and Hong, Y. (2003) Contribution of the zinc finger to zinc and DNA binding by a suppressor of posttranscriptional gene silencing. *J. Virol.* **77**, 696–700.

Vasu, S.K. and Forbes, D.J. (2001) Nuclear pores and nuclear assembly. *Curr. Opin. Cell Biol.* **13**, 363–375.

Vetter, G., Hily, J.M., Klein, E., Schmidlin, L., Haas, M., Merkle, T. and Gilmer, D. (2004) Nucleo-cytoplasmic shuttling of the beet necrotic yellow vein virus RNA-3-encoded p25 protein. *J. Gen. Virol.* **85**, 2459–2469.

Voinnet, O. (2001) RNA silencing as a plant immune system against viruses. *Trends Genet.* **17**, 449–459.

Wagner, R.R. (1987) Rhabdovirus biology and infection. In *The Rhabdoviruses* (Wagner, R.R., ed.), pp. 9–74. New York: Plenum Press.

Wagner, J.D.O., Choi, T.J. and Jackson, A.O. (1996) Extraction of nuclei from sonchus yellow net rhabdovirus-infected plants yields a polymerase that synthesizes viral mRNAs and polyadenylated plus-strand leader RNA. *J. Virol.* **70**, 468–477.

Wagner, J.D.O. and Jackson, A.O. (1997) Characterization of the components and activity of sonchus yellow net rhabdovirus polymerase. *J. Virol.* **71**, 2371–2382.

Waigmann, E., Ueki, S., Trutnyeva, K. and Citovsky, V. (2004) The ins and outs of non-destructive cell-to-cell and systemic movement of plant viruses. *Crit. Rev. Plant Sci.* **23**, 195–250.

Wang, Y., Tzfira, T., Gaba, V., Citovsky, V., Palukaitis, P. and Gal-On, A. (2004) Functional analysis of the *Cucumber mosaic virus* 2b protein: pathogenicity and nuclear localization. *J. Gen. Virol.* **85**, 3135–3147.

Ward, B.M. and Lazarowitz, S.G. (1999) Nuclear export in plants. Use of geminivirus movement proteins for a cell-based export assay. *Plant Cell*, **11**, 1267–1276.

Weidman, M.K., Sharma, R., Raychaudhuri, S., Kundu, P., Tsai, W. and Dasgupta, A. (2003) The interaction of cytoplasmic RNA viruses with the nucleus. *Virus Res.* **95**, 75–85.

Wen, W., Meinkoth, J.L., Tsien, R.Y. and Taylor, S.S. (1995) Identification of a signal for rapid export of proteins from the nucleus. *Cell*, **82**, 463–473.

van Wezel, R., Liu, H., Tien, P., Stanley, J. and Hong, Y. (2001) Gene *C2* of the monopartite geminivirus *Tomato yellow leaf curl virus-China* encodes a pathogenicity determinant that is localized in the nucleus. *Mol. Plant–Microbe Interact.* **14**, 1125–1134.

Whittaker, G.R. and Helenius, A. (1998) Nuclear import and export of viruses and virus genomes. *Virology*, **246**, 1–23.

Whittaker, G.R., Kann, M. and Helenius, A. (2000) Viral entry into the nucleus. *Annu. Rev. Cell Dev. Biol.* **16**, 627–651.

Wozniak, R.W. and Lusk, C.P. (2003) Nuclear pore complexes. *Curr. Biol.* **13**, R169.

Yang, Q., Rout, M.P. and Akey, C.W. (1998) Three-dimensional architecture of the isolated yeast nuclear pore complex: functional and evolutionary implications. *Mol. Cell*, **1**, 223–234.

Yelina, N.E., Erokhina, T.N., Lukhovitskaya, N.I., Minina, E.A., Schepetilnikov, M.V., Lesemann, D.E., Schiemann, J., Solovyev, A.G. and Morozov, S.Y. (2005) Localization of *Poa semilatent virus* cysteine-rich protein in peroxisomes is dispensable for its ability to suppress RNA silencing. *J. Gen. Virol.* **86**, 479–489.

Zenklusen, D. and Stutz, F. (2001) Nuclear export of mRNA. *FEBS Lett.* **498**, 150–156.

Zupan, J., Citovsky, V. and Zambryski, P.C. (1996) *Agrobacterium* VirE2 protein mediates nuclear uptake of ssDNA in plant cells. *Proc. Natl Acad. Sci. USA*, **93**, 2392–2397.