Herpesviruses have a well-defined replication phase within the nucleus, where they are known to exploit many of the cellular processes performed there. Upon virus entry into the host cell, the viral DNA genome is directed into the nucleus by an as-yet-undefined mechanism and is subsequently transcribed and replicated by a combination of host cell machinery and virus genes products (1, 17, 22). At later stages in the replication cycle, assembly of the herpesvirus particle is initiated within the nucleus as the newly replicated virus DNA genome is packaged into assembling capsids (39, 41). As a consequence, herpesviruses must target several classes of their gene products, including transcription factors, DNA replication factors, scaffold proteins, and capsid proteins, to the nucleus. A number of virus proteins, such as the immediate-early proteins ICP0 (13, 30) and ICP27 (19, 28), the DNA replication protein encoded by gene UL9 (27), and the capsid protein VP19C (40), have been shown to contain classical nuclear localization signals (NLSs), which are defined in the primary amino acid sequence of these proteins (15, 32). Such NLS-containing proteins are translocated from the cytoplasm into the nucleus through the nuclear pores, a process mediated by cellular proteins typified by the heterodimeric complex of importin α and β proteins (15, 32). Thus, transient expression of these proteins in isolation from other virus products is sufficient to allow their localization to the nucleus.

However, in many cases protein localization observed by transient expression of individual virus genes does not correlate with the subcellular targeting of the same proteins during virus infection, and there are several examples of virus proteins which lack recognizable NLSs but which are nonetheless directed to the nucleus during virus infection. Several proteins have been shown to piggyback into the nucleus via an interaction with an NLS-containing partner either of viral origin, as is the case with the capsid proteins VP5 (31, 40) and VP23 (40), or of cellular origin, as has been suggested for the transactivator of immediate-early gene expression VP16, which appears to be directed into the nucleus by the cellular protein HCF (25). Thus, herpesviruses may employ a range of nuclear targeting mechanisms to ensure the correct cellular compartmentalization of their gene products.

The herpes simplex virus (HSV) structural protein VP22 is a major component of the virion tegument (18, 23, 42), that is, the virus compartment located between the capsid and the envelope (4). The role of VP22, which is encoded by gene UL49 (11), is unclear, but it does not contain a recognizable NLS, thereby suggesting that VP22 would not be targeted to the nucleus by the classical pathway during virus infection. We have previously studied the subcellular localization of VP22 using immunofluorescence of transiently transfected cells and have shown that, consistent with the lack of an NLS, transiently expressed VP22 localizes primarily in the cytoplasm of expressing cells (5, 6). However, our studies of transient transfection also revealed the presence of VP22 in the nuclei of a subpopulation of cells (5), but we have demonstrated that the majority of cells containing such nuclear VP22 have obtained it by an unusual process which we have termed intercellular spread (5). In this situation, VP22 is taken up into nonexpressing cells either from neighboring cells or from cell culture medium and is transported rapidly and efficiently to the nucleus (5). Thus, while transiently expressed VP22 clearly exhibits differential subcellular localization, this appears to be determined by whether or not the cell is expressing VP22 (in which case it is cytoplasmic) or has taken up the protein (in which case it is nuclear). Furthermore, we have also recently addressed the issue of VP22 targeting during infection by constructing an
HSV type 1 (HSV-1) recombinant virus expressing green fluorescent protein (GFP)-tagged VP22 in place of wild-type VP22 (8). Time-lapse analyses of cells infected with this virus showed that, in cells infected at high multiplicity, GFP-22 was detected in a predominantly cytoplasmic location throughout the virus life cycle (8). Therefore, our results from virus infection suggest that, at high multiplicity at least, VP22 localizes primarily in the cytoplasm. However, the issue of VP22 compartmentalization is complicated by a number of other reports which suggest that VP22 may, in certain circumstances, localize within the nucleus during infection. For example, several biochemical studies have indicated that VP22 may be present in the nuclear fraction of infected cells (23, 35), while some infected-cell immunofluorescence studies have led to the proposal that VP22 may be targeted to the nucleus at certain stages of the HSV-1 replication cycle (29, 37).

Nevertheless, these latter results are somewhat ambiguous, with one report suggesting that VP22 is nuclear at early times in infection and cytoplasmic later (29), while another recent study suggested that VP22 was cytoplasmic at early times and moved to the nucleus at later times during infection (37). These previous observations on the complexity of VP22 localization, and its potential for different subcellular patterns, led us to use GFP-tagged VP22 expressed either by transient transfection or by virus infection to investigate further the trafficking and compartmentalization of VP22 in cells actively expressing the protein, rather than cells which have taken up the protein by intercellular spread. In this report, we demonstrate that GFP-22 expressed in isolation localizes in several different patterns ranging from exclusively cytoplasmic to exclusively nuclear. Using time-lapse analysis of GFP-22-expressing cells, we have further shown that these patterns represent VP22 localization at different stages of the cell cycle. Thus, the initial expression of GFP-22 in an interphase cell results in the cytoplasmic concentration of the protein, with no GFP-22 fluorescence detectable in the nucleus. However, upon entry into mitosis, the previously cytoplasmic GFP-22 becomes tightly associated with mitotic chromatin and remains bound there throughout cell division and subsequent nuclear membrane reformation. Moreover, once located in the nucleus, GFP-22 appears to be retained there, resulting in an exclusively nuclear pattern of GFP fluorescence throughout the G1 stage of the cell cycle. Thus, GFP-22 translocation from the cytoplasm to the nucleus of cells expressing the protein is regulated entirely by the process of mitosis. Furthermore, we have demonstrated the same pathway of M-phase nuclear translocation and retention using the GFP-22-expressing virus. Moreover, multiplicity of infection appears to influence this pathway, as it is observed principally in cells infected at low multiplicity, when the timing of the virus replication cycle may allow a greater proportion of infected cells to traverse cell division. Thus, we demonstrate an alternative and unusual mechanism for nuclear entry by a normally cytoplasmic virus protein and show that such translocation is an independent feature of VP22 which does not require the presence of additional virus proteins. The function of such differential cell-cycle-dependent localization of a herpesvirus tegument protein remains to be determined.

**RESULTS**

**Localization of transiently expressed GFP-22 in live cells.** To initially characterize GFP-VP22 localization in expressing cells, COS-1 cells were transiently transfected with an expression vector for the GFP-22 fusion protein, and expression was allowed to continue for up to 48 h after transfection. At various times after transfection, the cells expressing the GFP-22 fusion protein were examined live and variations in the fluorescence patterns were compared to those observed for unfused GFP. At all times, the localization pattern of unfused GFP was the same, with the protein localizing in a diffuse cytoplasmic and nuclear pattern (Fig. 1A). By contrast, at least five GFP-VP22 patterns were identified which were markedly different from that observed for unfused GFP (Fig. 1B to F). At 24 to 36 h posttransfection, the predominant pattern of VP22 localization was that of thick cytoplasmic filaments (Fig. 1B) similar to those previously observed by immunofluorescence of fixed cells expressing VP22 (5, 6) and which we have shown to represent the reorganization and bundling of microtubules by high levels of expressed VP22 (6). However, at earlier times posttransfection, a large number of cells contained GFP-22 either in a diffuse cytoplasmic location (Fig. 1C), or in a diffuse cytoplasmic pattern on which was superimposed a small number of filaments (Fig. 1D). The likely explanation for the early diffuse patterns of GFP-22 localization is that they are the precursors of the extensive filamentous pattern shown in Fig. 1B. Strikingly, in all these cases GFP-22 was detectable only within the cytoplasm, with no detectable fluorescence in the nucleus, suggesting that, unlike GFP, the GFP-22 protein was retained within the cytoplasm of the cell (Fig. 1; compare panel A with panels B, C, and D).

However, at later times of up to 48 h posttransfection two very different patterns of localization were observed in a subpopulation of GFP-22-expressing cells (Fig. 1E and F). The first of these clearly depicts cells in the process of mitosis, with GFP-22 specifically bound to condensed chromosomes (Fig. 1E), and while the example shown here is a cell in an early stage of mitosis, i.e., prometaphase, cells at all stages of mitosis were easily identified within the population of transfected cells. The second pattern consisted of GFP-22 localized specifically in the nucleus, with little or no fluorescence detectable in the...
cytoplasm (Fig. 1F). In these cases, the fluorescent nuclei always occurred in doublets.

**Microtubule bundling by VP22 results in a block to cell division.** The diverse range of GFP-22 localization patterns identified during transfection, together with the binding of GFP-22 to mitotic chromatin, led us to speculate that GFP-22 compartmentalization may in some way be related to the cell cycle. Thus, to investigate the ultimate fate of cells expressing GFP-22, we initiated time-lapse analyses of COS-1 cells containing GFP-22 in its various locations. Cells grown on a coverslip were transfected with the expression vector for GFP-22, and 20 h later, the coverslip was transferred to a microscope chamber on a heated stage and allowed to equilibrate for 30 min. A suitable cell containing, in the first instance, filamentous GFP-22 (similar to Fig. 1B) was selected for further observation, and images were collected every 10 min over a period of 24 h. The resulting time-lapse analysis was processed to produce both an animation (found as Fig. 2 at [http://www.mcri.ac.uk/VirusAssembly/timelapse.html](http://www.mcri.ac.uk/VirusAssembly/timelapse.html)) and a gallery of 2-hourly time points (Fig. 2). Between 2 and 4 h later, an increase in the concentration of bundles around the nucleus was observed (Fig. 2, 2 hrs and 4 hrs) while 6 h from the start of the analysis, the microtubule bundles began to contract into the perinuclear region (Fig. 2, 6 hrs), eventually becoming localized in a large mass at the side of the nucleus (Fig. 2, e.g., 10 hrs). The pattern of VP22 in this cell did not alter over a

![Figure 1](http://www.mcri.ac.uk/VirusAssembly/timelapse.html) Subcellular localization of GFP-22 expressed by transient transfection. COS-1 cells grown in a two-well coverslip chamber were transfected with either plasmid pEGFPC1 (A) or plasmid pGE155 (B to F), expressing GFP or GFP-22, respectively. The cells were examined live by confocal microscopy at 28 (A and B), 16 (C and D), or 44 (E and F) h after transfection.
further period of 12 h, during which time a normal cell would have been expected to progress through mitosis. Taken together with a number of other time-lapse analyses which we have conducted (data not shown), we believe that this animation demonstrates that microtubule bundling by GFP-22 inhibits cell division.

**Soluble GFP-22 translocates from the cytoplasm to the nucleus during mitosis.** As the above data indicate that cells containing VP22-induced microtubule bundles were unable to progress through mitosis, we next addressed the possible sources of GFP-22 responsible for the protein binding to mitotic chromatin. In having observed a large number of cells expressing GFP-22, it was clear that we never found individual nuclei containing the fluorescent protein, only nuclei in pairs, suggesting that GFP-22 may localize in its nuclear pattern only after cell division. Thus, to investigate events immediately prior to the onset of mitosis, we wished to carry out a time-lapse analysis on GFP-22-expressing cells as they traversed the

![Figure 2: The fate of VP22-induced microtubule bundles in transiently transfected cells.](http://www.mcri.ac.uk/VirusAssembly/timelapse.html)
G2/M boundary of the cell cycle. COS-1 cells were transfected with the GFP-22 expression vector as described above and transferred to the heated chamber, and cells were examined for the characteristic appearance of rounding up, indicating the start of M phase. A single cell, which at the beginning of the time series contained diffuse cytoplasmic GFP-22, which had the appearance of a cell entering mitosis, was selected (0'), and images were collected every 2 min for a total time of 80 min. Representative images are shown in the gallery, and the corresponding animation can be found elsewhere (http://www.mcri.ac.uk/VirusAssembly/timelapse.html).

Further analysis, images were then collected every 2 min for a total of 80 min, and the resulting time series has been presented as an animation (found at http://www.mcri.ac.uk/VirusAssembly/timelapse.html) and as a gallery of selected images (Fig. 3). Over the first 10 min of the time series, the pattern of diffuse cytoplasmic GFP-22 was unaltered (Fig. 3, 0' and 10'). However, by 14 min a large amount of fluorescence had ap-

FIG. 3. Translocation of GFP-22 from cytoplasm to nucleus during cell division. COS-1 cells grown on 42-mm-diameter coverslips were transfected with the expression vector for GFP-22 and transferred to a heated chamber 24 h later. A single cell containing diffuse cytoplasmic GFP-22, which had the appearance of a cell entering mitosis, was selected (0'), and images were collected every 2 min for a total time of 80 min. Representative images are shown in the gallery, and the corresponding animation can be found elsewhere (http://www.mcri.ac.uk/VirusAssembly/timelapse.html).
peared within the nucleus, suggesting that at least some of the GFP-22 had translocated from the cytoplasm to the nucleus (Fig. 3, 14'). Two minutes later, the mitotic chromatin had condensed and was entirely decorated with bound GFP-22 (Fig. 3, 16'). Mitosis then progressed normally (see also Fig. 4), with GFP-22 remaining bound to the individual chromosomes throughout metaphase (Fig. 3, 40' and 48') and anaphase (Fig. 3, 64'). These results suggest then that diffuse cytoplasmic GFP-22 (Fig. 1C) progresses into chromatin-bound GFP-22 (Fig. 1F) at the start of mitosis.

To determine the fate of such mitotic cells with GFP-22 bound to condensed chromatin, we carried out time-lapse

FIG. 4. Chromatin-associated GFP-22 is retained in the nucleus after cell division. COS-1 cells were treated as described in the legend to Fig. 3, and a cell in the early stages of mitosis was selected for further analysis (0'). Images of this cell were collected every minute for 150 min, with 10-min intervals presented in the gallery. The corresponding animation can be found elsewhere (http://www.mcri.ac.uk/VirusAssembly/timelapse.html).
analysis of a mitotic cell beginning at an early stage of mitosis, in which GFP-22 was already outlining the condensed chromosomes. Images were collected every minute for a total of 160 min, and a time series was produced from the resulting images, which is presented as both an animation (found as Fig. 4 at http://www.mcri.ac.uk/VirusAssembly/timelapse.html) and a gallery of representative static images (Fig. 4). At the first time point (Fig. 4, 0'), the GFP-22-expressing cell was already in the early stages of mitosis, with the fluorescent chromosomes localizing in a prometaphase pattern. Over the next 30 min, the chromosomes became more organized into a metaphase pattern, until by 60 min after the start of the time lapse, the two sets of sister chromatids had pulled apart through anaphase (Fig. 4, 60'). Over the next 40 min, the now fluorescently decorated chromosomes decondensed, the nuclear membrane appeared to reform on the two daughter nuclei, and the cell underwent cytokinesis, at which time two daughter cells became apparent (Fig. 4, 100'). The time lapse was carried out for a further 60 min, during which time the GFP-22 fluorescence remained in the nucleus and showed no sign of returning to the cytoplasm. These results demonstrate that the double-nucleus pattern of GFP-22 localization (Fig. 1F) is the consequence of cell division and that the mitotic pattern of GFP-22 (Fig. 1E) progresses into the nuclear pattern of GFP-22 (Fig. 1F). Therefore, the process of mitosis alters the compartmentalization of GFP-22 from a cytoplasmic to a nuclear location.

Heterogeneous localization of GFP-22 in HSV-1-infected cells. Our previous studies of cells infected with HSV-1 expressing GFP-22 had suggested that during virus infection VP22 was exclusively cytoplasmic (8). However, most of these results were obtained from high-multiplicity infections in which the virus replication cycle would be relatively rapid, reducing the possibility of infected cells progressing through cell division. We therefore reasoned that, to identify infected cells either in mitosis or postdivision, infection would have to be carried out at a low multiplicity to ensure that cells were infected with single infectious particles. Thus, Vero cells were infected with the GFP-22-expressing virus (166v) at a multiplicity of 0.001, and infection was allowed to progress for 36 h. The cells were then examined for foci of infection which were the consequence of virus spreading from individual infected cells into neighboring cells, thereby capturing infected cells over a range of stages of the replication cycle. As observed before in high-multiplicity infections (8), a large number of these infected cells contained GFP-22 in an exclusively cytoplasmic location. However, we consistently observed smaller numbers of cells on the edges of these centers of infection which contained GFP-22 in either its mitotic localization (Fig. 5A and B, arrowed) or its double nuclear location (Fig. 5B and C, arrowed). Furthermore, in addition to these patterns we also frequently observed GFP-22 in a single nuclear localization (Fig. 5D, arrowed), a pattern which we had never observed during transient expression of the protein.
These results indicated that infection with the GFP-22-expressing virus at low multiplicity enabled us to detect GFP-22 in a pattern other than its predominant cytoplasmic pattern. To investigate this in more detail, a similar experiment was conducted whereby Vero cells were infected at a multiplicity of 0.001, but in this case the cells were examined at 16 h postinfection to analyze the range and ratio of infected-cell GFP-22 patterns. At this early stage, we were able to detect GFP-22 in four different patterns, namely, cytoplasmic (Fig. 6A), mitotic (Fig. 6B), double nuclear (Fig. 6C), and single nuclear (Fig. 6D). Moreover, the percentage of each of these patterns, with 44% being cytoplasmic, 2% being mitotic, and 54% being nuclear, seemed to approximately reflect the number of cells which should have progressed through cell division over a 16-h period.

GFP-22 translocation at M phase occurs in HSV-1-infected cells. To confirm that the progression of mitotic cell-bound GFP-22 in infection was the same as that observed in the transiently transfected cells, we carried out time-lapse analyses on a range of infected mitotic cells, the results of which are summarized in Fig. 7. While a number of these animations demonstrated that infected GFP-22 behaved in a manner similar to that of transiently expressed GFP-22 (Fig. 7, pathway 1), we also found a range of alternative fates for these mitotic cells, which all seemed to be the result of aberrant mitoses. In particular, we observed that the single nuclear pattern shown in Fig. 6D was also a result of mitosis, but in this case the condensed chromatin did not separate into two populations but decondensed within a single nucleus (Fig. 7, pathway 3). In addition, there were a number of cells which progressed with a normal mitosis but failed to undergo cytokinesis, resulting in cells containing two fluorescent nuclei (Fig. 7, pathway 2). Last, we have evidence that some infected mitotic cells underwent an extremely prolonged mitosis (up to 16 h) before dividing as normal (Fig. 7, pathway 4). Thus, while the underlying process of GFP-22 translocation from the cytoplasm to the nucleus is a feature of both transiently expressing and infected cells, the end result of infected cells in mitosis is complicated by these cells exhibiting a range of cell division defects.

DISCUSSION

In this paper, we demonstrate that the herpesvirus tegument protein VP22 exhibits cell-cycle-dependent compartmentalization. We have exploited GFP technology to track the fate of individual GFP-22-expressing cells and to elucidate the VP22 trafficking pathway through the cell cycle. This trafficking begins with the protein localizing exclusively to the cytoplasm of the expressing cell during interphase. In a subpopulation of transiently expressing cells, this cytoplasmic VP22 binds cellu-
lar microtubules, resulting in their bundling and stabilization. In this situation, mitosis appears to be inhibited by the increased stabilization of the microtubule network (Fig. 8, pathway 1), a feature which is common to the overexpression of many other microtubule-stabilizing proteins (2, 21, 26, 34, 38). However, in another population of VP22-expressing cells, VP22 remains diffuse in the cytoplasm and upon entry into mitosis immediately associates with the condensed cellular chromatin where it remains throughout M phase and into G1 of the next cell cycle. Cytoplasm-to-nucleus translocation of VP22 is therefore a consequence of cell division (described in Fig. 8, pathway 2). The result of such differential compartmentalization is that at any time in a population of nonsynchronized expressing cells, VP22 can be found in a range of patterns, including exclusively cytoplasmic, exclusively nuclear, or both nuclear and cytoplasmic. Moreover, the chromatin interaction of VP22 at mitosis ensures that VP22 is transferred into the two daughter cells, providing an efficient mechanism for equal protein distribution through cell division. Thus, in transiently transfected cells expressing VP22 there are two potential outcomes dictating VP22 localization—either the cells proceed through mitosis, in which case VP22 translocates from the cytoplasm to the nucleus, or the cells are blocked for mitosis by microtubule stabilization and VP22 remains in the cytoplasm associated with the microtubule network (summarized in Fig. 8). While we do not yet understand the mechanism which determines whether VP22 binds microtubules or whether it remains diffuse in the cytoplasm, we would speculate that it is due to either the overall concentration of VP22 in the cytoplasm or the timing of VP22 expression and accumulation with respect to the cell cycle. In a similar manner, our studies on the intracellular trafficking of GFP-22 in HSV-1-infected cells have shown that there are also two potential outcomes of virus infection dictating the localization of VP22 (Fig. 8), which are likely to be determined by the cell cycle stage of the host cell at the time of infection. While the mitosis-dependent cytoplasm-to-nucleus translocation of VP22 also occurs during infection and is primarily observed in cells infected at low multiplicity (Fig. 8, pathway 2), the more frequent outcome involves the completion of the entire virus replication pathway before the cells can enter mitosis, resulting in the restriction of VP22 localization to the cytoplasm of the infected cell (Fig. 8, pathway 3).

In this paper, we have addressed the issue of VP22 intracellular trafficking in cells which are expressing the protein, rather than in cells into which the protein has spread (5). We have previously shown that the efficiency of GFP-22 intercellular movement is lower than that of unfused VP22 (5), to the extent that GFP-22 spread cannot be detected in live cells expressing the fused protein and often requires antibody enhancement for detection (7). Thus, our live-cell analysis and time-lapse animations detect only the initial GFP-22-expressing cell and not the surrounding cells which have taken up the protein. However, taken together with our previous observations on intercellular trafficking, the results presented here suggest that VP22 uses two quite distinct pathways to enter the nucleus—either the mitosis-dependent pathway of the expressing cell or the mitosis-independent pathway of the nonexpressing cell, confirming that VP22 exhibits a complex range of cellular localization and trafficking pathways.

The finding of this unusual intracellular trafficking property of VP22 in cells expressing the protein provides an explanation for at least some of the variations in VP22 localization observed in previous reports (29, 37). In particular, a recent report by Pomeranz and Blaho (37) used immunofluorescence to show VP22 in the nuclei of cells located toward the exterior of virus plaques, in the same manner as we observed in our live cells infected with the GFP-22-expressing virus (demonstrated in Fig. 5). Moreover, as the cell fixation techniques used for immunofluorescence are known to cause a loss of a large percentage of mitotic cells which are only weakly attached to the substrate, the mitotic patterns of VP22 could easily have been missed in this previous study. However, it is clear that the mechanism of VP22 translocation to the nucleus during cell division could not be responsible for the apparent accumulation of the protein in the nuclei of all infected cells, as described previously for either early times in infection (29) or late times in infection (37). While there is evidently a discrepancy...
between these two previous reports concerning the timing and extent of VP22 localization to the nucleus, our own model based on the live-cell studies described here, suggesting that VP22 is almost exclusively cytoplasmic until cell division, differs from both scenarios. Thus, there may still be several issues concerning VP22 localization during virus infection which need to be addressed.

The vast majority of proteins which are targeted to the nucleus do so by the use of classical NLSs, consisting of clusters of basic residues which associate with cellular proteins to translocate them through the nuclear pores (15, 32). Some cellular proteins, such as basal transcription factors, appear to be targeted to the nucleus constitutively, while others are targeted only when their NLSs are somehow activated, for example, by phosphorylation (14, 16, 43). By contrast, the cell-cycle-regulated nuclear translocation of VP22 does not seem to involve the classical nuclear import pathway, as there are no consensus NLS sequences within the VP22 open reading frame. However, VP22 compartmentalization and translocation are strikingly similar to those of the cellular protein cyclin B1. During interphase, cyclin B1 is observed exclusively in the cytoplasm, where it appears to localize to microtubules (20, 36). However, at the start of M phase and prior to nuclear envelope breakdown, cyclin B1 is phosphorylated at its amino terminus, resulting in the creation of a nuclear import signal followed by the rapid translocation of the protein from the cytoplasm to the nucleus (16). Once there, cyclin B1 is observed in close association with condensed chromatin and remains bound to the dividing chromatin until anaphase, when the protein is destroyed. While we have previously identified two phosphorylation sites within the VP22 open reading frame which are substrates for cellular kinases (9, 10), we have not yet investigated the cell cycle characteristics of these sites. Furthermore, we do not yet know if VP22 moves into the nucleus before or after nuclear envelope breakdown. While the role of VP22 nuclear translocation and subsequent chromatin binding during virus infection is as yet unclear, it is interesting to speculate that it may be functioning in a similar manner to that of cyclin B1 and participating in the regulation of mitosis in infected cells. It is also noteworthy that while the chromatin labeling by GFP-22 in infected cells revealed a range of aberrant mitoses (Fig. 7), these effects were not caused by VP22 but as shown previously are likely to be due to binding of the immediate-early protein IE110 to chromosome centromeres (12).

Although VP22 is a major structural component of the tegument, it is unlikely that the nuclear localization of VP22 described here is required for the protein to be incorporated into the virus particle, as we have previously demonstrated GFP-22 trafficking during infection at high multiplicity in which GFP fluorescence was detected exclusively in the cytoplasm (8). VP22 translocation during mitosis may therefore be indicative of a function separate from its role during virus assembly and in addition to its properties of both microtubule stabilization (6) and intercellular transport (5), which we have previously described. Thus, as is the case for the tegument protein VP16, which performs roles as both a transactivator protein (3, 24, 33) and an essential structural protein (18, 44), it would seem that VP22 contributes multiple functions to the virus replication cycle.

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