Cellular Protein WDR11 Interacts with Specific Herpes Simplex Virus Proteins at the trans-Golgi Network To Promote Virus Replication

Kathryne E. Taylor, Karen L. Mossman

Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, Institute for Infectious Disease Research, McMaster University, Hamilton, Ontario, Canada

ABSTRACT
It has recently been proposed that the herpes simplex virus (HSV) protein ICP0 has cytoplasmic roles in blocking antiviral signaling and in promoting viral replication in addition to its well-known proteasome-dependent functions in the nucleus. However, the mechanisms through which it produces these effects remain unclear. While investigating this further, we identified a novel cytoplasmic interaction between ICP0 and the poorly characterized cellular protein WDR11. During an HSV infection, WDR11 undergoes a dramatic change in localization at late times in the viral replication cycle, moving from defined perinuclear structures to a dispersed cytoplasmic distribution. While this relocation was not observed during infection with viruses other than HSV-1 and correlated with efficient HSV-1 replication, the redistribution was found to occur independently of ICP0 expression, instead requiring viral late gene expression. We demonstrate for the first time that WDR11 is localized to the trans-Golgi network (TGN), where it interacts specifically with some, but not all, HSV virion components, in addition to ICP0. Knockdown of WDR11 in cultured human cells resulted in a modest but consistent decrease in yields of both wild-type and ICP0-null viruses, in the supernatant and cell-associated fractions, without affecting viral gene expression. Although further study is required, we propose that WDR11 participates in viral assembly and/or secondary envelopment.

IMPORTANCE
While the TGN has been proposed to be the major site of HSV-1 secondary envelopment, this process is incompletely understood, and in particular, the role of cellular TGN components in this pathway is unknown. Additionally, little is known about the cellular functions of WDR11, although the disruption of this protein has been implicated in multiple human diseases. Therefore, our finding that WDR11 is a TGN-resident protein that interacts with specific viral proteins to enhance viral yields improves both our understanding of basic cellular biology as well as how this protein is co-opted by HSV.

With worldwide seroprevalence rates reaching 60 to 90% (1), herpes simplex virus 1 (HSV-1) is a tremendously successful human pathogen. HSV-1 particles consist of a double-stranded DNA genome encased by an icosahedral capsid, surrounded by a proteinaceous layer known as the tegument, which is in turn enclosed by an envelope of host-derived lipids (2). During the lytic replication cycle, viral glycoprotein-mediated fusion of the envelope with the host cell plasma membrane releases the capsid and tegument proteins into the cytosol (3). HSV capsids are then transported to the nucleus along microtubules via molecular motor proteins (4). The release of the genome into the nucleus is followed by viral gene expression in a sequential manner, beginning with the immediate-early (IE) genes and then progressing to the early (E) and late (L) classes, resulting in viral genomic DNA replication and packaging into capsids.

Although subsequent steps have been controversial, it is now generally accepted that capsids escape from the nucleus by first budding from the inner nuclear membrane, acquiring a primary envelope, and then fusing with the outer nuclear membrane, releasing the unenveloped capsids into the cytoplasm (5, 6). To become mature particles, these capsids must then undergo secondary envelopment. Although the exact mechanism and subcellular location at which this event takes place have been highly debated (7–12), current evidence appears to support the trans-Golgi network (TGN) as the major site of secondary envelopment (8, 9, 13–17). This network of tubules connected with the trans-face of the Golgi apparatus functions in the secretory pathway as a sorting station, directing cargo into distinct carriers which transport them to their final cellular destinations (reviewed in references 18 and 19). Although the exact details of secondary envelopment remain unclear, interactions between the tegument proteins and glycoproteins that gather at the TGN and the capsid and capsid-associated tegument proteins are thought to help drive this process (5, 20). The resulting enveloped viral particles within transport vesicles travel to and fuse with the plasma membrane, releasing mature viruses, via the classical pathway of cargo transport from the TGN to the cell surface (17). Infected cell protein 0 (ICP0) is a multifunctional IE HSV-1 protein with roles in enhancing viral gene expression during lytic replication, promoting reactivation from latency, and opposing both the intrinsic and the interferon-mediated antiviral response (reviewed in reference 21). Traditionally, the majority of ICP0 functions have been thought to occur in the nucleus—where the
protein localizes early in infection (22)—through its ability to target proteins for degradation via the proteasome using the E3 ubiquitin ligase activity of its RING finger domain (23–25). However, ICP0 is found largely in the cytoplasm as the infection progresses (26–30), and evidence is mounting that it also has important activities in this location (31–33). Interestingly, a RING finger–independent cytoplasmic function for ICP0 in promoting viral replication has recently been suggested (33), implying that this protein may have activities that are distinct from its function in proteasome-mediated protein degradation. However, the mechanism through which ICP0 produces this effect remains unclear.

Although widely expressed in human tissues (34) and highly conserved in vertebrates (35), the cellular protein WD repeat domain 11 (WDR11) is poorly understood. Disruption of the WDR11 gene has been found in both human glioma cells (34) and breast cancer cells (36), leading to the suggestion that WDR11 may act as a tumor suppressor, and mutations in WDR11 have also been reported in patients with idiopathic hypogonadotropic hypogonadism (IHH), Kallmann syndrome (KS), and combined pituitary hormone deficiency (CPHD) (37), conditions characterized by low sex steroids and delayed puberty (35, 38). Additionally, depletion of WDR11 was found to sensitize cells to the AB-type toxin ricin (39). Although a potential function in autophagy for WDR11 has been proposed to explain some of these results (39, 40), the molecular mechanisms through which WDR11 is involved in these diverse activities remain unclear.

To further characterize the mechanism of action of ICP0 in the cytosol, we recently performed quantitative mass spectrometry to identify proteins that commmunoprecipitated with ICP0 from cytoplasmic extracts (unpublished data). One of the proteins identified in this screen was WDR11. Here, we characterize the ICP0-WDR11 interaction in more detail. We found that WDR11 has a tight perinuclear distribution in uninfected human fibroblasts, but undergoes a profound redistribution at late times postinfection in an HSV-1–specific manner. Interestingly, although ICP0 was found to interact strongly with WDR11, this protein is not degraded during an HSV-1 infection, and the relocation of WDR11 was found to occur independently of ICP0. To begin to explain this finding, we show for the first time that WDR11 is localized to the TGN and interacts specifically with some, but not all, HSV-1 virion components. Additionally, depletion of WDR11 was found to cause a modest but reproducible decrease in the yields of both cell-associated and secreted virus for wild-type HSV-1 as well as an ICP0-null mutant, without affecting viral gene expression, suggesting a possible role for this protein in viral assembly in the cytoplasm or secondary envelopment.

**MATERIALS AND METHODS**

Cells, viruses, reagents, and plasmids. Human embryonic lung (HEL) fibroblasts and U2OS osteosarcoma cells were purchased from the American Type Culture Collection (ATCC) and grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin. Polyinosinic/poly(C) (poly I·C; GE Healthcare) was directly added to cul-
Statistical analysis. Statistical analysis was performed using GraphPad Prism. Where necessary, values were first adjusted via logarithmic transformation to equalize variances.

RESULTS

ICP0 interacts with the cellular protein WDR11. In a screen for cytoplasmic proteins that bind to ICP0, we recently identified the poorly characterized cellular protein WDR11. To further investigate this potential interaction, we first performed reciprocal co-immunoprecipitations (co-IPs) in cells infected with wild-type HSV-1 strain 17 syn (Fig. 1A). Accordingly, endogenous WDR11 was confirmed to be present after IP with an anti-ICP0 antibody, and ICP0 was found after IP with an anti-WDR11 antibody. Identical results were also observed when cells were infected with D8/FXE (data not shown), a virus encoding an ICP0 mutant lacking both the RING finger of ICP0 as well as the nuclear localization signal, which causes the protein to localize to the cytoplasm (33). We next tested whether ICP0 induces the proteasome-mediated degradation of WDR11, given that the RING finger is not required for this interaction, by determining the levels of WDR11 after infection (Fig. 1B), and we found no loss of WDR11, even at late times postinfection with either 17 syn, which expresses high levels of ICP0 at this time, or the ICP0-null HSV-1 strain dl1403.

WDR11 becomes relocalized at late times postinfection in an HSV-specific but ICP0-independent manner. We next examined the subcellular localization of endogenous WDR11. In primary human HEL fibroblasts (Fig. 2A), WDR11 is found in the cytoplasm in a characteristic pattern adjacent to the nucleus in mock-treated cells. However, beginning approximately 8 h after infec-

FIG 1 ICP0 interacts with WDR11 but does not cause its degradation. (A) HEL cells were infected with the indicated viruses at 10 PFU/cell for 8 h, and then samples were harvested via cytoplasmic extract and immunoprecipitated with the indicated antibodies. Eluents were then analyzed via Western blotting for ICP0 and WDR11. (B) HEL cells were infected with the indicated viruses at 10 PFU/cell for 10 h and then harvested in RIPA extract and analyzed for WDR11, ICP0, and actin levels via Western blotting.

FIG 2 WDR11 has a distinct perinuclear distribution that becomes dispersed at late times after HSV-1 infection in an ICP0-independent manner. HEL cells (A) or U2OS cells (B) were mock treated or infected with the indicated viruses at 10 PFU/cell for the times shown and then fixed and analyzed for WDR11 localization via immunofluorescence. Nuclei were identified using Hoechst stain.
tion with 17 syn (data not shown) and becoming pronounced by 12 h postinfection (hpi), WDR11 undergoes a dramatic redistribution, losing the tight perinuclear localization and becoming dispersed throughout the cytoplasm. In contrast, even at the very late time point of 24 hpi in HEL cells, no redistribution of WDR11 was observed with dl1403, suggesting that this effect may be dependent on ICP0. However, because ICP0-null viruses show a replication defect in human fibroblasts at the MOI we used here, it was not clear whether the lack of WDR11 relocation was directly due to the absence of ICP0 or was rather a by-product of the decreased replication of dl1403. Therefore, the experiment was repeated in U2OS cells (Fig. 2B), which complement the growth of ICP0-null viruses (43). Although these highly permissive cells were beginning to show morphological changes as a result of the HSV-induced cytopathic effect at late times postinfection, the relocation of WDR11 was clearly detectable with dl1043 as well as 17 syn by 12 hpi, demonstrating that this effect correlates with the efficiency of viral replication and not specifically with the expression of ICP0.

Given that WDR11 relocation was not dependent on ICP0 expression, we next examined whether this change in localization was a general consequence of viral infection or was due to activation of the antiviral response (Fig. 3A). HEL cells were therefore infected with HCMV, VSV-GFP, or SeV at MOIs adjusted to produce approximately equal levels of cytopathic effect at 16 hpi, or the cells were treated with the synthetic double-stranded RNA analogue poly I·C. At that time, WDR11 redistribution was observed only after infection with 17 syn and remained intact with all other treatments. While this time point is late in the replication cycles of SeV, VSV, and HSV (44–48), it occurs relatively early for HCMV (49). Therefore, we also monitored WDR11 localization over 4 days after infection with HCMV (data not shown), and although we never observed a dispersal of WDR11 comparable to that seen with HSV-1, we did find a subtle but consistent relocalization of WDR11, in a pattern similar to the previously reported reorganization of TGN markers into HCMV assembly sites (50).

To begin to determine how HSV-1 produces this effect on WDR11, we next investigated whether the dispersal could be prevented by blocking viral DNA synthesis using phosphonomoacetate (PAA) (Fig. 3B). As expected, this resulted in the inhibition of viral late gene expression, as monitored by observing gC production, and also prevented the redistribution of WDR11, implicating one or more late gene products in this process.

WDR11 colocalizes with the TGN marker TGN46. This HSV-1 late gene-specific dispersal of WDR11 in the cytoplasm at late times postinfection is similar to the disruption of both the Golgi apparatus and the TGN that has been reported during HSV infections (8, 15, 51–54). Indeed, markers for both structures have been shown to lose their tight perinuclear organization and become scattered throughout the cytoplasm at approximately 8 to 12 h after infection (15, 52). Therefore, we wondered if WDR11 might in fact be a TGN component, becoming redistributed upon the dispersal of the TGN during HSV infection. To investigate this, we examined the localization of WDR11 relative to the TGN marker TGN46 via immunofluorescence. In uninfected cells (Fig. 4A), the two proteins showed strong colocalization, suggesting that WDR11 does in fact reside in the TGN. During 17 syn infection (Fig. 4B), the relocation of WDR11 mirrored the dispersal of TGN46, although the two proteins did not maintain their colocalization upon breakdown of the TGN. This demonstrated, for the first time, that WDR11 is a TGN component. To further confirm this observation, we next treated the cells with brefeldin A (BFA), which causes the collapse of TGN membranes (55), and we found that this treatment also caused the relocalization of WDR11 into distinctive perinuclear spots that continued to colocalize with TGN46 (Fig. 4C), verifying that WDR11 resides in the TGN. We also observed strong colocalization of WDR11 and gE at early times postinfection, when gE has been previously shown to localize to the TGN (54), but not at later times, when gE moves to cell-cell junctions (data not shown).

WDR11 interacts with specific additional HSV-1 proteins. Although WDR11 is neither relocated nor degraded by ICP0, the strong interaction between these two proteins suggests that
WDR11 may have a role in HSV-1 replication. To begin to examine this further, we investigated whether additional viral proteins interacted with WDR11 via co-IP in HEL cells (Fig. 5A). Intriguingly, WDR11 was found to bind to gB, VP16, and VP5 in addition to ICP0, but not to ICP4, ICP27, gC, or gE, suggesting that it interacts specifically with some, but not all, HSV-1 proteins. However, it was possible that the proteins not detected after WDR11 IP are simply expressed at lower levels than the interacting proteins. To rule out this possibility, we repeated this experiment in U2OS cells (Fig. 5B), which allow very high levels of expression of viral proteins. As these cells permit efficient replication of dl1403, we also determined whether ICP0 was required for the interaction of the various viral proteins with WDR11. A similar pattern was observed after WDR11 IP with U2OS cells compared to HEL cells, with the exception of ICP27, which was now observed to interact with WDR11. However, despite abundant expression, neither gC, gE, nor ICP4 was found in the eluents, confirming our observations that WDR11 interacts only with specific viral proteins. Interestingly, there was no change in the proteins found to interact with WDR11 in the presence or absence of ICP0. It is important to note that no gC expression was detected after infection with dl1403, consistent with the recent observation that this virus contains an unintentional secondary mutation disrupting the gC gene (56).

FIG 4 WDR11 colocalizes with the TGN marker TGN46. HEL cells were mock treated (A), infected with 17 syn at 10 PFU/cell for 10 h (B), or treated with 10 μg/ml BFA or the vehicle control for 30 min (C) and then fixed and analyzed for TGN46 and WDR11 localization via immunofluorescence.
Since ICP0 expression is not necessary for ICP27, gB, VP16, or VP5 to bind to WDR11, we were interested in determining whether ICP0 itself requires another viral protein to bridge its interaction with WDR11. To investigate this, we performed the WDR11 IPs in U2OS cells transfected with pCI-D8 (57), which encodes a mutant form of ICP0 lacking its nuclear localization signal, as exogenous wild-type ICP0 expressed in the absence of other viral proteins is restricted to the nucleus (26, 58), where it may not have access to WDR11. Interestingly, we found that ICP0 is capable of interacting with WDR11 in the absence of all other viral proteins, demonstrating that while ICP0 is sufficient to bind to WDR11, it is not necessary for the binding of the other viral proteins, suggesting that multiple independent interactions occur between WDR11 and HSV-1 virion components.

WDR11 depletion reduces viral yields without decreasing viral gene expression. To further investigate the potential role of WDR11 in HSV-1 replication, we used siRNA to deplete WDR11, using U2OS cells to permit a comparison of the effect of WDR11 knockdown on the growth of both 17 syn and dl1403. Cells depleted of WDR11 remained healthy, showing no changes in morphology or growth rate (data not shown). While siRNA treatment reduced WDR11 levels in U2OS cells, depletion was not complete, which was particularly evident in infected cells (Fig. 6A). We next examined the expression of IE (ICP27), E (ICP8), and L (gC) proteins after infection with 17 syn in control cells and WDR11 siRNA-treated cells (Fig. 6C). No detectable change in the levels of any of these proteins was observed after depletion of WDR11, suggesting that any possible function of this protein in the viral replication cycle is downstream of virus entry and gene expression. We also did not observe any change in the localization of ICP0, ICP4, gB, gC, gE, VP5, or VP16 via immunofluorescence in cells treated with WDR11 siRNA compared to the control-treated cells after 10 h of infection with 17 syn (data not shown), suggesting that WDR11 is not involved in directing the intracellular targeting of viral proteins.

To investigate this further, cells treated with control or WDR11 siRNA were subsequently infected with either 17 syn or dl1403, and cell-associated or secreted virus was separately quantified after 24 h (Fig. 6B). Interestingly, reducing WDR11 levels caused a modest but reproducible decrease in both supernatant and cell-associated virus, equally for 17 syn and dl1403. No such decrease in titer was observed for VSV (data not shown), demonstrating that the loss of WDR11 specifically impacts HSV replication and does not result from nonspecific effects on cell health or density. Therefore, WDR11 appears to promote HSV replication in an ICP0-independent manner. Since the equal decrease in both secreted and cell-associated virus appears to rule out a function for WDR11 in egress itself, these data, taken together, suggest a potential role for WDR11 in viral assembly.

**DISCUSSION**

Currently, the function of the cellular protein WDR11 remains unclear. A clue to its possible role is that it contains a series of loosely conserved motifs known as WD repeat domains, making it a member of the WD repeat family of proteins. These repeats fold to form a propeller-like arrangement, with each WD repeat resulting in a blade in a circular structure (59–61). Despite their shared sequence motif and structure, WD repeat proteins have a great deal of functional diversity, and the WD repeats themselves do not exhibit any catalytic activity (62). Instead, the common function of members of the WD repeat family seems to be the coordination of multiprotein complexes, with the propeller structure providing a stable scaffold for several simultaneous protein–protein interactions (62, 63). Indeed, WDR11 has been suggested to bind to multiple proteins, including STAT3 (64), EMX1, Tagln2, Ndr4g4, Nrxn3, and Hey1 (35), C17orf75 (39), and UBXD7 (65), although the significance of these interactions requires further study.

In this work, we identified WDR11 as a novel binding partner for ICP0. At first glance, this interaction is particularly intriguing, because it does not result in the loss of the WDR11 protein. Traditionally, it was thought that proteins binding to ICP0 are targeted for proteasome-mediated degradation (66–79). However, several binding partners have been identified that are not directed to the proteasome by ICP0 (27, 80–88). This is consistent with our recent observation that ICP0 has two distinct cytoplasmic functions, neither of which involve protein degradation; first, it blocks...
antiviral signaling via a mechanism that requires the RING finger but not the proteasome, and second, that it promotes viral replication in a RING-independent manner (35). The exact mechanisms behind the nondegradative effects of ICP0 remain to be fully characterized.

Although ICP0 interacts strongly with WDR11, its relocation during HSV infection is not dependent on ICP0. Instead, WDR11 relocation correlates with efficient viral replication. This finding is analogous to what has been observed with cyclin D3 and IFI16, ICP0-binding proteins that were initially reported to be stabilized or degraded, respectively, in an ICP0-dependent manner (78, 80). However, when conditions were adjusted to equalize replication of the ICP0-null and wild-type viruses, the alleged “ICP0-mediated” effects were observed to occur in the absence of ICP0 (89, 90). This highlights the importance of considering viral replication levels during ICP0-null infection.

Here, we have shown for the first time that WDR11 is a TGN-resident protein and that its redistribution is a result of the virus-induced fragmentation of the Golgi apparatus and TGN (8, 15, 51–54). Previous descriptions of WDR11 localization have been variable. Endogenous WDR11 in neuroblasts was reported to be cytoplasmic, although the precise subcellular distribution was not determined (35), while exogenous, GFP-tagged WDR11 was shown to have either a diffuse cytoplasmic distribution in U2OS cells (35) or a punctate perinuclear localization in HeLa cells which partially colocalized with the autophagosome marker LC3 (39). LC3 can associate with the TGN membrane during autophagy (91), consistent with our observations. It is currently unknown whether the dispersal of the Golgi apparatus and TGN is a specific effect of HSV late proteins to promote egress (52) or simply a by-product of envelopment (92), as neither inhibiting nor augmenting the fragmentation of the Golgi apparatus impacts HSV replication in cell culture (51). Similarly, we observed that late gene expression is required for WDR11 dispersal, although whether this is a direct or indirect effect is unclear. Regardless, our observed relocation of WDR11 with similar kinetics to the dispersal of TGN46 further substantiated the idea that WDR11 is a TGN component, as did our observation that disruption of the TGN with BFA also caused the relocation of WDR11.

Collectively, our data suggest that HSV recruits WDR11 for optimal assembly or secondary envelopment, in an ICP0-independent manner. Although the effect of WDR11 depletion of virus production was modest, the knockdown of WDR11 was not complete. Moreover, observations in cultured cells do not always reflect the biological outcome in an animal. For example, although HSV-1 lacking the virion host shutoff protein vhs replicates to wild-type levels in cultured cells, it is highly attenuated in mice (93). Similarly, depletion of STAT1 does not affect HSV-1 replication in cell culture (94), but it renders mice highly susceptible to HSV infection in vivo (95–100). Furthermore, high levels of WDR11 are found in the brains of embryonic and adult mice (35), and disruptions to WDR11 are associated with IHH/KS/CPHD (35, 38), conditions that result from abnormal migration of specific neurons during embryogenesis (reviewed in reference 101). Additionally, WDR11 interacts with dysbindin, a neuronal protein enriched in synapses (102), as well as EMX1 (35), a protein with important functions in the developing nervous system (reviewed in reference 103). Therefore, it is possible that the function of WDR11 may be more crucial for viral replication in vivo in neurons as opposed to cells of the periphery. Indeed, the pathway to assembly and egress in neurons is thought to require specialized adaptations, as a result of the long distances between the cell body and the axon termini over which HSV virions must be transported (reviewed in reference 104). Unfortunately, the current lack of a WDR11 knockout mouse, potentially due to embryonic lethality (35), precludes directly addressing the in vivo relevance.
Perhaps the most intriguing of our observations is that WDR11 not only interacts with ICP0 but also with specific viral proteins, including gB, VP16, and VP5, but not ICP4, gC, or gE. It is well-known that virion components form an intricate meshwork, with tegument proteins binding to one another as well as to the cytoplasmic tails of envelope glycoproteins on one side and the capsid proteins on the other (reviewed in reference 6). The specificity of WDR11 binding suggests that it selectively targets particular viral proteins and that our results cannot simply be explained by the complex protein-protein interactions among virion components. The basis for this selectivity is currently unclear. For example, both ICP0 (105) and ICP4 (106) are tegument components, and indeed, these two proteins have been found to interact directly (107), but only ICP0 binds WDR11. Similarly, both gC and gB are major virion glycoproteins (108), and yet only gB interacts with WDR11. Like gC, minor virion component gE does not bind WDR11, despite its early localization to the TGN (54, 109). VP16 is a major tegument component (108), and interestingly, it has been shown to associate with gB but not gC (110). The interaction of WDR11 with major capsid protein VP5 is intriguing, as it suggests that WDR11 not only binds specific tegument and envelope proteins but also the capsid itself. Since VP5 is a component of the outer shell of the capsid and has been shown to be accessible for interactions with tegument components (111–115), it may also be available for binding to WDR11. ICP27 has been reported to be packaged in virions grown on BHK cells (116), and not those grown on Vero cells (105, 117), suggesting that the incorporation of this protein may be cell type dependent, consistent with our observations that ICP27 interacts with WDR11 in U2OS but not HEL cells. Our results also fit with reports that gC and gB are independently incorporated into the virion (118), and likewise that ICP4 is packaged independently of ICP0 (119). However, further study is clearly necessary to determine the basis for the ability of WDR11 to discriminate between specific viral proteins.

Given that WDR11 both localizes to the TGN, a major site of secondary envelopment (8, 9, 13–17), and specifically interacts with several HSV virion components, and further that it is a member of a protein family involved in coordinating multiple simultaneous protein-protein interactions, we propose that this protein plays a role in HSV morphogenesis. Although WDR11 has not been found to be among the known cellular proteins incorporated into mature HSV virions (120), our hypothesis is supported by our observations that WDR11 depletion decreases viral yields without affecting viral gene expression, while the fact that both secreted and cell-associated viruses were reduced after WDR11 knockdown suggests that this protein could be functioning in reenvelopment as opposed to egress. Although the exact mechanism is not yet clear, secondary envelopment is thought to be driven by a complex series of protein-protein interactions between glycoproteins associated with the TGN membrane, tegument components, and capsid proteins (10, 121–144)—a process that could be coordinated through the actions of WDR11. However, transmission electron microscopy (TEM) did not reveal any striking differences in virion morphogenesis after 17 syn infection of cells treated with either control or WDR11 siRNA (data not shown). Although this could indicate that WDR11 is not involved in viral assembly, it is more likely that TEM, while useful for identifying gross defects in secondary envelopment or egress, is not appropriate for detecting the subtle differences expected given the modest decrease in the viral titer observed in the cells depleted of WDR11.

The role of cellular proteins in the reenvelopment process remains poorly characterized. The ESCRT (endosomal sorting complex required for transport) machinery, which has a multitude of roles in processes involving membrane curvature and fission (reviewed in reference 145), has been implicated in HSV envelopment, with Vps24/CHMP3 (10) and Vps4 (10, 11) suggested to perform the physical budding step. Moreover, inhibiting the ESCRT-III complex has also been reported to block secondary envelopment (146). In addition, several Rab GTPases have been implicated in HSV reenvelopment. Depletion of Rab1 and Rab43 results in decreased viral assembly, although this may be partially explained by indirect effects, such as decreased processing of glycoproteins and their impaired transport to the TGN in the absence of Rab1 and extensive disruption to the TGN structure upon depletion of Rab43 (147). Similar to what we observed with WDR11, Rab27a, which is involved in exocytosis and membrane trafficking (148), has been found to colocalize with HSV proteins at the TGN and increase viral yields and has therefore been suggested to play a role in HSV morphogenesis and/or egress (149).

Altogether, this work identifies WDR11 as a TGN resident protein that interacts specifically with certain HSV-1 virion components to augment viral yields, leading us to propose that this protein plays a role in viral assembly and/or secondary envelopment. Although further study is necessary to confirm this hypothesis, this work provides new insights into both the function of a poorly characterized cellular protein as well as the incompletely understood mechanism of HSV-1 secondary envelopment.

ACKNOWLEDGMENTS

This work is supported by Canadian Institutes of Health Research (CIHR) grant MOP-57669. K.T. was supported by a Natural Sciences and Engineering Research Council (NSERC) Alexander Graham Bell Canada graduate doctoral scholarship.

We thank P. Ezzati and K. Coombs for technical and intellectual contributions.

REFERENCES

28. Henaff

25. Lopez


48. Smith JD, De Harmon E. 1973. Herpes simplex virus and human cyto-


51. Avitabile E, Di Gaeta S, Torrisi MR, Ward PL, Roizman B, Cam-

padelli-Fiume G. 1995. Redistribution of microtubules and Golgi appar-


55. Reaves B, Banting G. 1992. Perturbation of the morphology of the


57. Everett RD, Meredith M, Orr A. 1999. The ability of herpes simplex

virus type 1 immediate-early protein Vmw110 to bind to a ubiquitin-


65. Chelbi-Alix MK, de The H. 1999. Herpes virus induced proteasome-


