Effect of Guanine Nucleotide Exchange Factor Inhibitors on pU34 Localization in Cells Infected with Herpes Simplex Virus Type-1

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Abstract

Herpes simplex virus type-1 (HSV-1) buds from the nucleus into the inner nuclear membrane (INM), where it forms enveloped capsids in the perinuclear space. Viral protein U34 knockouts in the INM, where it creates and co-localizes with other viral and cellular proteins and is required for viral budding at the INM. To further elucidate this role, we measured HSV-infected cells with BFA and observed U34 localization in the nucleus by immunofluorescence and confocal microscopy. Preliminary results show that infected cells treated with BFA or Tyrophostin AG 1478 display a mislocalization of pU34 at the INM, suggestive of a connection between a smooth distribution of the protein in infected cells that were not treated with BFA. Future aspects of this project will focus on specific targets of BFA, such as Arf1p. This study is relevant because there are U34 homologues in all herpesvirus family members and there are no known homologues in cellular genus. It is, therefore, an obvious potential target for pharmaceutical intervention.

Background

Herpesviruses:

The herpesvirus family includes numerous viruses that infect both animals and humans. There are many common features across the members. They all have dsDNA (approximately 100 kbp) with an RNA transcription step in the nucleoplasm. They share an icosahedral capsid structure and a lipoidal envelope. Clinical features in humans are typically oral ulcers or vesicular eruptions. Infection persists in neuronal ganglia. Infection persists in neurons and can cause seizures long after primary infection clears.

HSV-1 Egress Pathway:

HSV viral protein U34 is required for HSV egress from the nucleus (all sequenced herpesviruses have U34 genes homologues), pU34 is made in the ER and then recruited to the inner nuclear membrane (INM). It co-localizes with other viral and cellular proteins related to envelopment. Enveloped capsids bud through the INM into the perinuclear space. INM enclosed capsids face the OMM for de-envelopment. Virion then moves from ER to Golgi network and exit-vesicles from the cell.

Materials & Methods

Viral stock of HSV-1/J strain was grown in Vero cells. HEP-2 cells were plated at 75% confluency on glass coverslips in 6-well plates with 2 coverslips per well. Cells were incubated at an multiplicity of infection (MOI) of 10 and visualized for 14 hours. Brefeldin A (BFA) treatment was performed 3 hours post-infection (hpi) at 37°C and 4 µM, respectively. Cells were fixed at 20°C, rinsed, and treated with 40 mM NH4Cl. Two blocking steps were performed (10% human/10% goat serum in PBS, 10% Brefeldin on PBS). FITC, primary marker (400U anti-diphtheria) was tagged to e-Lamin α/β. Texas Red red marker (1:100 anti-mouse) was tagged to e-Lamins α/β. Fluorescence was visualized with an Olympus IX71 confocal microscope.

Conclusions

1. pU34 at the INM
2. pU34 knock out
3. HEP-2 cells infected with HSV-1 and treated with BFA or Tyrophostin AG 1478 display an accumulation of U34 at the INM as well as puncta throughout the cytoplasm. Uninfected cells show a smooth distribution of U34 around the nuclear membrane.

References

