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Defects in Cytoplasmic Assembly and Sorting of US9 Pseudorabies Virus Mutants

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Alpha-herpes viruses such as herpes simplex virus (HSV) and pseudorabies virus (PRV) rely on anterograde transport from sensory ganglia, down neuronal axons, to reach epithelial tissues and spread [1]. Transport is achieved by tethering the alpha-herpes virus to kinesin motors which move along microtubule tracks in the axon [1]. The role of two viral proteins, gE/gI and US9, in this mechanism is still unclear. One model indicates that the gE/gI and US9 proteins promote tethering of PRV virus particles onto kinesin motors [2]. Another model argues that gE/gI and US9 do not participate in transport and are instead active in viral assembly and sorting within the cytoplasm [3,4]. Since PRV gE/gI and US9 mutants have yet to be screened for cytoplasmic defects, one PRV mutant lacking gE/gI and US9 and another only lacking US9 were characterized via transmission electron microscopy (TEM). We observe that PRV mutants for gE/gI and US9 exhibit defective viral assembly and sorting which may indicate that the main function of the gE/gI and US9 proteins is in the cytoplasmic steps of anterograde transport, rather than kinesin-mediated axonal transport.

Two cell lines were infected with wild type PRV and PRV mutants for gE/gI and US9. Differentiated CAD cells which are a derivative of mouse catecholaminergic central nervous systems cells were selected because of their demonstrated susceptibility to alpha-herpes virus infection [3]. Around 10% of the CAD cells we observed exhibited no axons and thus may not have been fully differentiated. The experiment was thus also repeated with rat hippocampal neurons purchased from Lonza Bioscience and ThermoFisher Scientific.

Cells were infected with one of three PRV strains; Becker, BaBe, or 161. PRV Becker is the wild-type virus. PRV BaBe contains deletions in gE/gI and US9. PRV 161 contains deletions only in US9. CAD cells were differentiated 7-10 days on glass cover slips before infection while rat hippocampal neurons were plated onto lysine/laminin-coated 13-mm Nunc™ Thermanox™ coverslips and incubated for 10-12 days. Both cell lines were infected for 10-12 hours using 5 PFU/cell. After incubation the cells were placed in Karnovsky's fixative and processed using a microwave assisted TEM protocol [5,6]. A Leica EM UC7 Ultramicrotome was used to produce 70 nm sections which were then counter-stained [7]. Samples were imaged at 120 kV on a FEI Tecnai™ Spirit TEM system and images were acquired using the AMT software interface on a NanoSprint12S-B cMOS camera system.

Wild type neurons of both cell types exhibited copious cell surface particles as well as less numerous cytoplasmic enveloped particles and unenveloped plasmid. In contrast, both BaBe and 161 mutants displayed relatively fewer cell surface particles and showed an accumulation of enveloped virions in the cytoplasm. Higher magnification images of both the wild type and mutant cell lines revealed a glut of malformed or misassembled virions in the mutant infected cells and relatively few in the wild-type infected cells. Both BaBe and 161 mutants showed statistically equivalent numbers of defects in viral assembly and sorting.

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The observed decrease in the trafficking of virus to the cell surface and subsequent increase in virus accumulation in the cytoplasm of cells infected with BaBe and 161 PRV mutants indicates that the gE/gI and US9 genes play a role in virus assembly and sorting. This bolsters the model outlined by Daniel, G *et al* [4] indicating that gE/gI and US9 are active in the cytoplasm and do not play a role in kinesin-mediated transport within axons [3, 8].

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