Assessing intergenomic recombination during HSV-1 infection and spread

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Overview

Herpes simplex virus type 1 (HSV-1) infection involves replication and spread in neurons, in part contributing to HSV-1 as the leading cause of viral encephalitis in developed countries. HSV-1 is a dsDNA virus with an accordingly low point mutation rate. Yet a high level of viral genome diversity has been observed across populations. Viral diversity is produced through the combined effects of evolutionary pressure and selection on HSV-1 genomes. We are establishing methods to quantify viral population diversity and the effects of evolutionary pressure on HSV-1 genomes. We quantified inter-viral genomic recombination using a marker transfer assay to understand the impact of selection pressures, such as neuron replication, on viral diversity. The marker transfer assay utilized two unique, comparably fit viral isolates with genetically distant fluorescent protein expressing cassettes to co-infect cells. We scored viral progeny as either parent or recombinant based on marker expression as a percentile of the population. We adapted the marker transfer assay for both in vitro and in vivo HSV-1 infections. We utilized the HSV murine eye model to quantify the effect of spread and replication in neurons on viral populations. Understanding how HSV responds to antiviral selections during replication and spread will enhance current treatment for herpes infection and provide insight for vaccine developments.

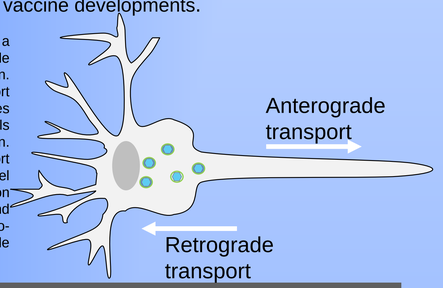

Figure 1. HSV is a neuroinvasive virus capable of axonal transportation. Retrograde axonal transport is when the viral particles travel from the cell body to the axon terminals to the cell body of the neuron. Anterograde axonal transport is when viral particles travel from the cell body to the axon terminal. It has been found that very few genomes co-infect cells during anterograde transport.

Quantification of HSV recombination with genetic marker transfer

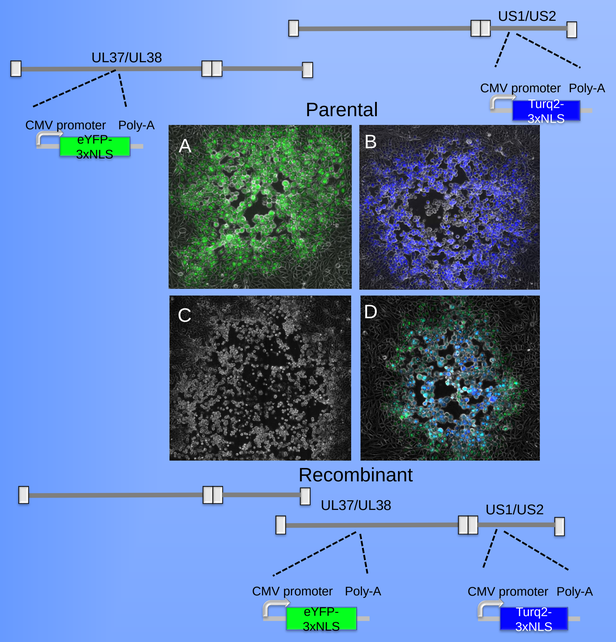

Figure 2. HSV genetic marker expression phenotype. (A) HSV OK12 with a YFP cassette between UL36/37. Plaques from this genome fluoresce yellow and represent a parent genome. (B) The novel HSV virus with a Turq2 cassette between US1/2. It is representative of a parent genome. Plaques fluoresce blue. (C) A recombinant phenotype that expresses no genetic mark and have no fluorescence. (D) A dual marker phenotype that expresses both genetic markers and fluoresces yellow and blue

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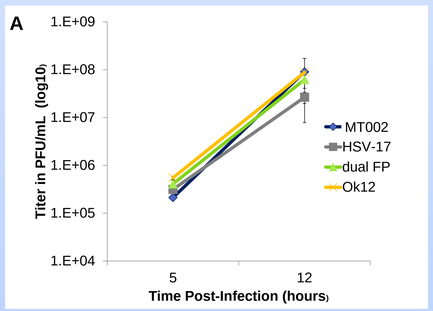
Characterization of novel viral isolate, MT002, and dual fluorescent recombinant



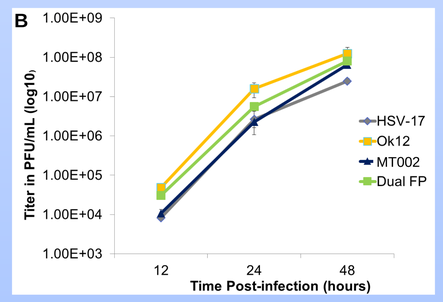
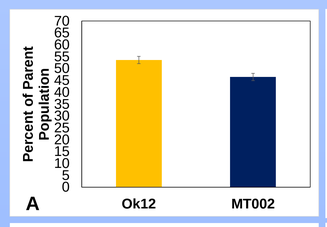
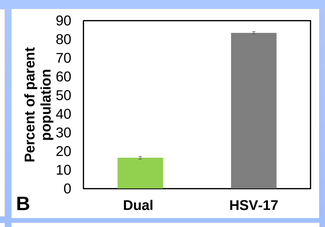
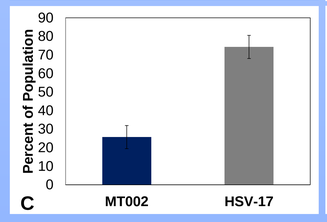


Figure 3. Single-step and multi-step viral replication experiments were performed to compare the replication capacity of marker isolates. (A) Single step growth curve illustrating the relative fitness of the viruses OK12 and MT002, HSV-17, and dual marker over the course of a single viral replication cycle. (B) Multi-step growth curve illustrating the relative fitness of viral isolates OK12, MT002, HSV-17, and the dual marker over several replication cycles.

Relative Fitness Comparisons







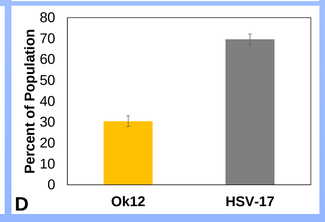


Figure 4. Direct and indirect fitness comparison assays were done between (A) MT002 and OK12 (B) HSV-17 and the dual recombinant (C) MT002 and HSV-17, (D) OK12 and HSV-17 to determine if viral isolates used are relatively fit. Equal MOI of two viruses as indicated were used to co-infect cells *in vitro.* Progeny was harvested, titered, and scored based on marker expression. Fitness was based on the portion of progeny population that each competing virus composed of (A) OK12 and MT002 are compose of similar portions of the population in a direct competition fitness assay. (B) The dual recombinant has a substantially less progeny composed of the population compared to HSV-17. (C) and (D) represent in-direct fitness comparison between MT002 and OK12 with HSV-17. In both (C) and (D) MT002 and OK12 make up less of the population than HSV-17, but both MT002 and OK12 make up similar portions of the total population in competition with HSV-17

Quantification of HSV recombination in immortalized cell line

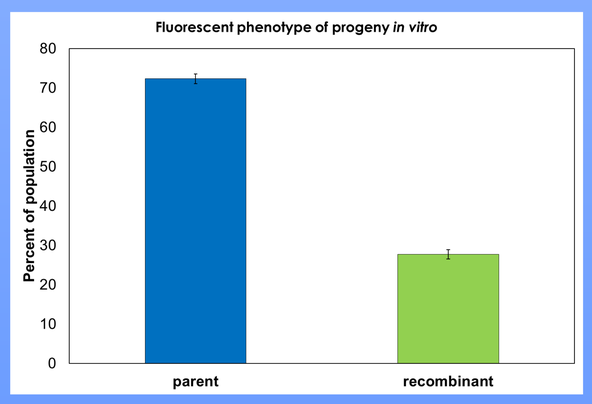


Figure 5. A recombination assay was performed in vero cells. MOI of 10 for both OK12 and MT002 were used to co-infect vero cells. The overall rate of recombination was 27.7% without any selective pressures with high infectious units of virus.

Effect of viral factors on recombination

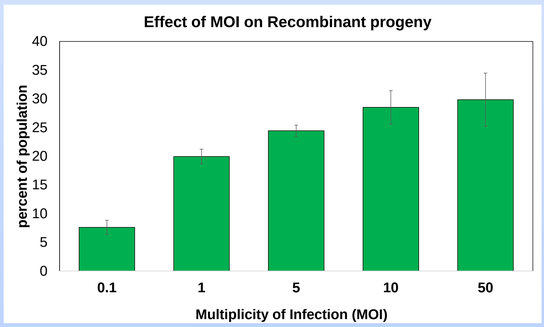


Figure 6. The effect of MOI on recombination frequency during initial infection with MT002 and OK12 in vero cells. MOI of 0.1, 1, 5, 10, 50 of OK12 and MT002 were used to co-infect cells.

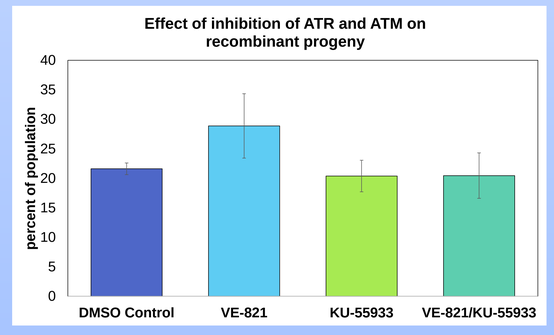


Figure 7. Frequency of recombinant progeny after inhibition of host H2AX phosphorylation using 100 nM VE-821 and 50 nM KU-55933 HSV-1 infection. VE-821 inhibits ataxia telangiectasia and rad3 related (ATR), KU-55933 inhibits ataxia telangiectasia mutated (ATM).

Quantification of HSV recombination in neuron chambers

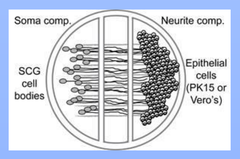
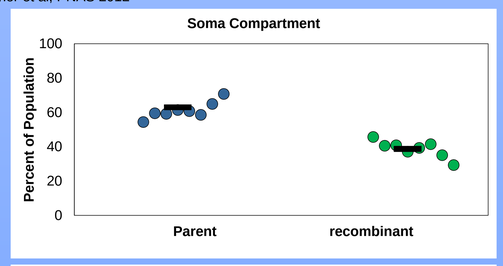


Figure 8. An *in vitro* model of compartmentalized neuronal cultures. In these cultures, dissociated superior cervical ganglia neurons (SCG) are plated in one compartment and axons are extended to a far compartment. Epithelial cells can be cultured on these isolated axons. This creates an environment where cell bodies are separated from the axons and epithelial cells.

Taylor et al, PNAS 2012



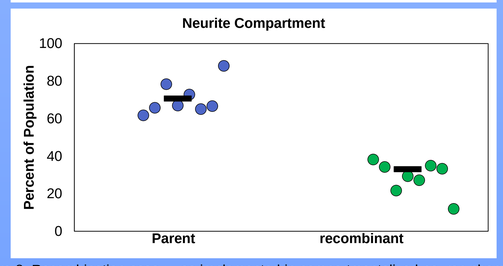


Figure 8. Recombination assay was implemented in compartmentalized neuron chambers. The soma compartment was inoculated with MT002 and OK12. Progeny from the soma and neurite compartment were harvested (A) The average recombination rate in the soma compartment was 38.6%. Each individual chamber has a recombination frequency similar to each other. (B) The average recombination in the neurite compartment is 33.0%. Each individual chamber had varying recombination frequency.

Acknowledgments

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Quantification of HSV recombination *in vivo*

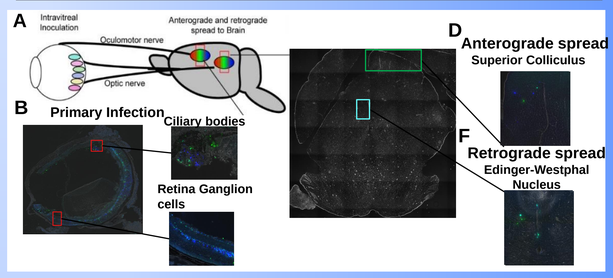
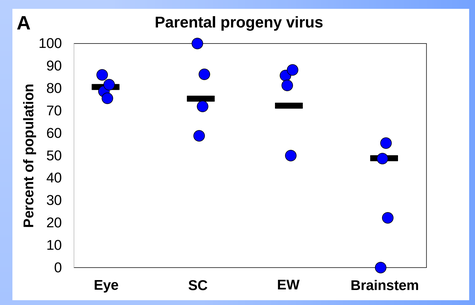


Figure 9. Murine eye model of HSV neuronal spread. A) Retinal ganglia cells and ciliary bodies are infected by an intra-vitreal injection of a mixture of HSV- OK12 and MT002. Initially infected retinal ganglia cells undergo high levels of co-infection and co-expression of the parent viruses. Infection spreads into the brain via two nerve tracts as illustrated, the Superior Colliculus (SC) following anterograde spread and the Edinger-Westphal Nucleus (EW) following retrograde spread. B) 72 hour post infection fluorescent images of the sectioned mouse eye. C) Sectioned mouse brain. Green box depict SC area on the sectioned sliced and the light blue box depicts area of the EW. Images of fluorescent protein expression were taken from brain sections in regions corresponding to the Superior Colliculus (D) and Edinger-Westphal Nucleus (F).



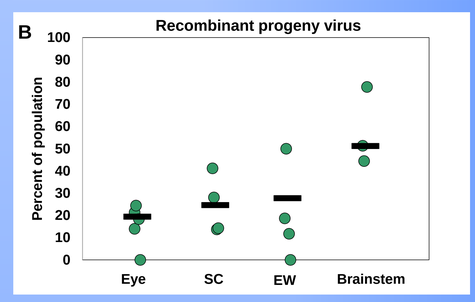


Figure 10. Frequency of (A) parental progeny and (B) recombinant progeny obtained from *in vivo*. Infected brains were dissected to isolate the brainstem, EW and SC along with the eye. The tissues were homogenized and progeny were plated on vero cells to determine fluorescence phenotype of progeny.

Conclusion

MT002 and OK12 were comparably fit relative to each other and do not impose a selective pressure during the recombination assay *in vitro.* The overall rate of recombination was 27.7% without any selective pressures with high infectious units of virus *in vitro*. The recombination frequency is dependent on the MOI of parent viruses. There appears to be little effect on the recombination rate after inhibition of ATR and ATM following infection. In compartmentalized neurons, the recombination frequency differed between the soma and neurite compartments but not greatly. The recombination frequency in the soma compartment had very little variance whereas the recombination frequency in the neurite compartment had high variance between individual chambers. This suggests there may be a selective pressure during anterograde transport. There was little difference between the recombination rate of the Superior Colliculus and the Edinger-Westphal Nucleus. However, there was high variance for these regions. The brainstem had an increased recombination rate, indicating that over several replication cycles and non-specific spread increased recombinant progeny. However, most of the recombinant progeny in the brainstem had no marker possibly indicating that there is a fitness deficit to our marked viruses *in vivo.*

Future work

We will continue to implement the recombination assay in vivo in a murine eye model to gain a better understanding of genome diversity generation. We will expand our exploration on the role of host H2AX phosphorylation and HSV recombination. Through quantifying mutations to the HSV genome, we hope to understand where viral genome diversity is generated, especially in a neuronal system