# HOST ESCRT RECRUITMENT BY STIV:
## effects of site directed mutagenesis of the major capsid protein

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## ABSTRACT

In eukaryotic cells, ESCRT (Endosomal Sorting Complexes Required for Transport) proteins are involved in membrane scission processes, such as cellular abscission and multivesicular body biogenesis. Interestingly, some archaeal organisms possess ESCRT-III homologs that are utilized during cell division. Some eukaryotic viruses, such as HIV and HCV, exploit ESCRT proteins for their egress from infected cells. It was recently discovered that the archaeal virus, *Sulfolobus* turreted icosahedral virus (STIV), interacts with the ESCRT components of its host, *Sulfolobus solfataricus*. We have shown a co-localization of STIV proteins and cellular ESCRT components at the site of viral egress. In addition, we have shown that the major capsid protein (MCP) of STIV (B345) interacts with one of the *Sulfolobus* ESCRT-III homologs. We hypothesize that B345 recruits the ESCRT-III (SSO0619) protein to intracellular vesicles that will eventually form the internal membrane of the assembled virion. Our studies have shown that the interaction of ESCRT-III and B345 occurs at the C-terminal end of B345. In addition, when B345 is expressed within *Sulfolobus* (in the absence of other viral proteins), it co-localizes with ESCRT components. We now want to determine the specific amino acids in the C-terminus of B345 that are required for the interaction with ESCRT-III. Single amino acid mutations within the C-terminal tail of B345 have been created via site-directed mutagenesis. The mutant forms of B345 will be expressed in *Sulfolobus* and tested for interactions with ESCRTs using western blots and pull-down assays. These results will help determine the details of how this archaeal virus interacts with its host. Overall, these findings will strengthen the evolutionary link between Eukarya and Archaea, and better inform the study of viruses that utilize ESCRTs.

## INTRODUCTION

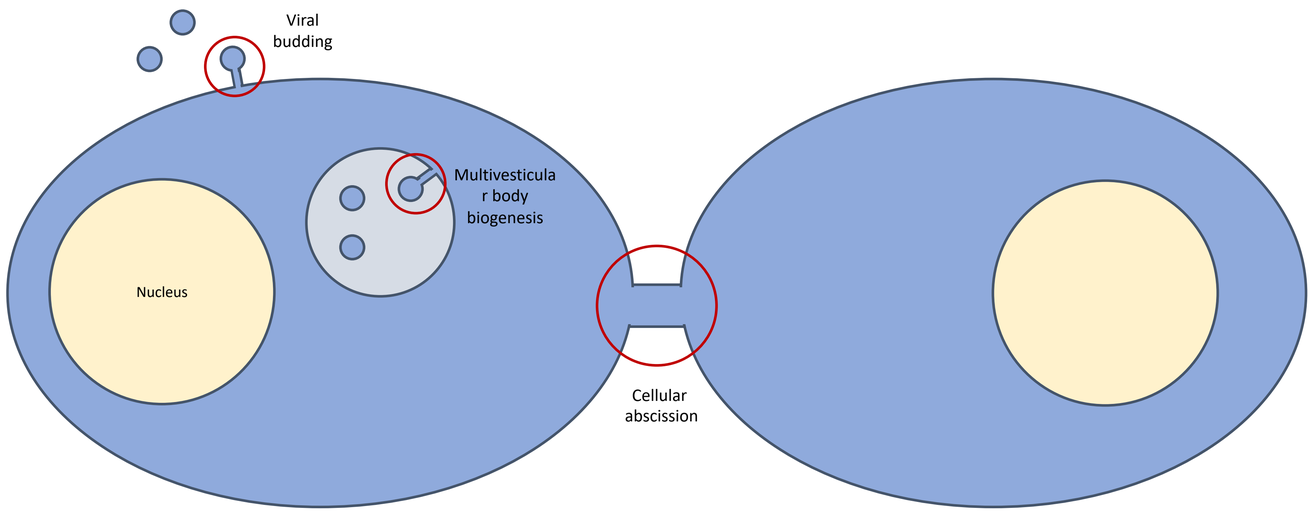
*Sulfolobus* turreted icosahedral virus (STIV) is an archaeal virus that infects *Sulfolobus solfataricus*. Although it has emerged as a model archaeal virus, its replication cycle has not been fully characterized. We have found that STIV exploits the Endosomal Sorting Complex Required for Transport (ESCRT) machinery of *S. solfataricus* in a similar fashion to the enveloped eukaryotic viruses, HIV and HCV, which suggests an evolutionary link between archaeal and eukaryotic viruses. Previous studies have shown that the Major Capsid Protein (MCP) of STIV (B345) interacts with an ESCRT-III homolog (SSO0619) in the *Sulfolobus* genome. This interaction was eliminated when the C-terminal 22 amino acids of B345 were deleted. We hypothesize that B345 interacts with ESCRT-III, such that it recruits the ESCRT-III protein to vesicles that will eventually become the internal membrane of the assembled virion. This project aims to determine the specific amino acids in the C-terminus of B345 that are required for its interaction with ESCRT-III.

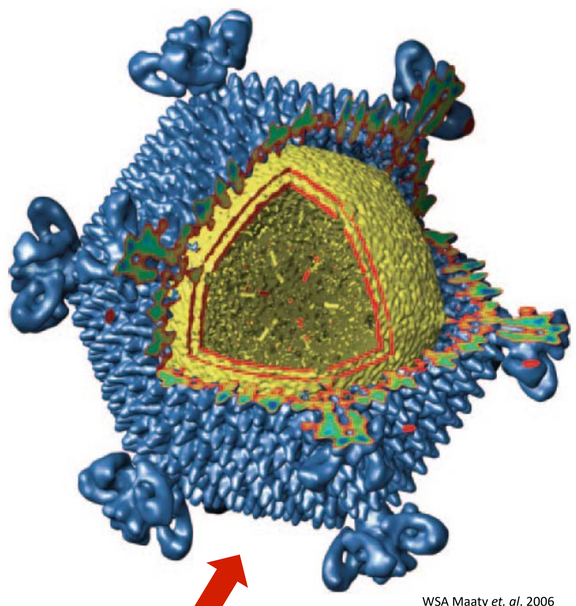
## METHODS

The V332D mutation within the C-terminal tail of B345 were created via site-directed mutagenesis at residues critical to STIV's interaction with ESCRT-III, and placed in the TOPO *E. coli* cloning vector using the restriction site ClaI and XmaI. Mutants were ligated into the *Sulfolobus* expression vector, pSeSD1, and sequenced by Source BioScience to verify the inserted sequence. Mutant forms of B345 will be expressed in *Sulfolobus* using an arabinose inducer. Production of mutant B345 proteins will be tested for through western blotting with anti-B345 antibody. The interaction between B345 and the ESCRT-III homolog will be tested using a GST pull-down assays.

## RESULTS/CONCLUSION

The V332D mutation has been created within the C-terminus of B345, and has been successfully ligated within the *Sulfolobus* expression vector, pSeSD1. Sequences of the ligation product have been sent for sequence verification. The results from this project will help determine the details of how STIV interacts with *Sulfolobus*, and strengthen the evolutionary link between Eukarya and Archaea.



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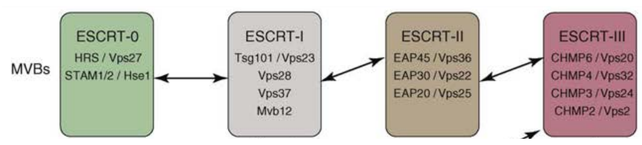
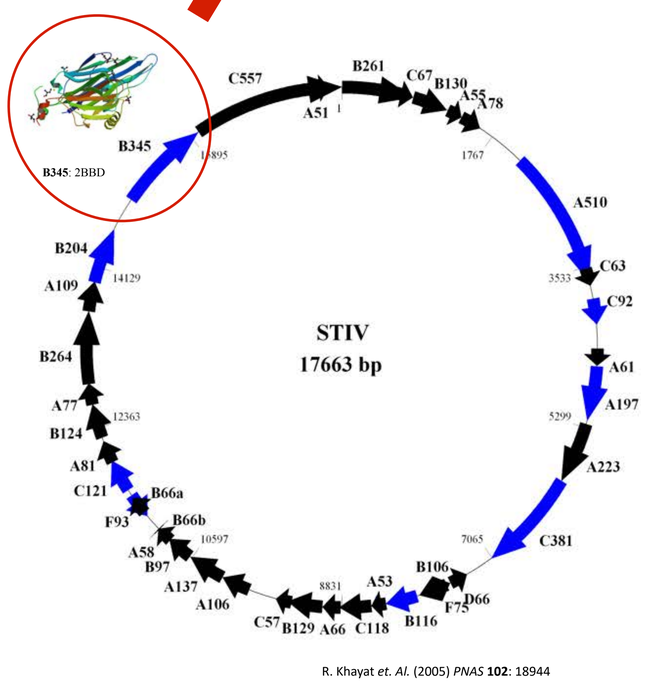



Modified from: RY Samson *et. al.* (2009) *Trends Microbiol.* **17**: 507.




R. Khayat *et. Al.* (2005) *PNAS* **102**: 18944

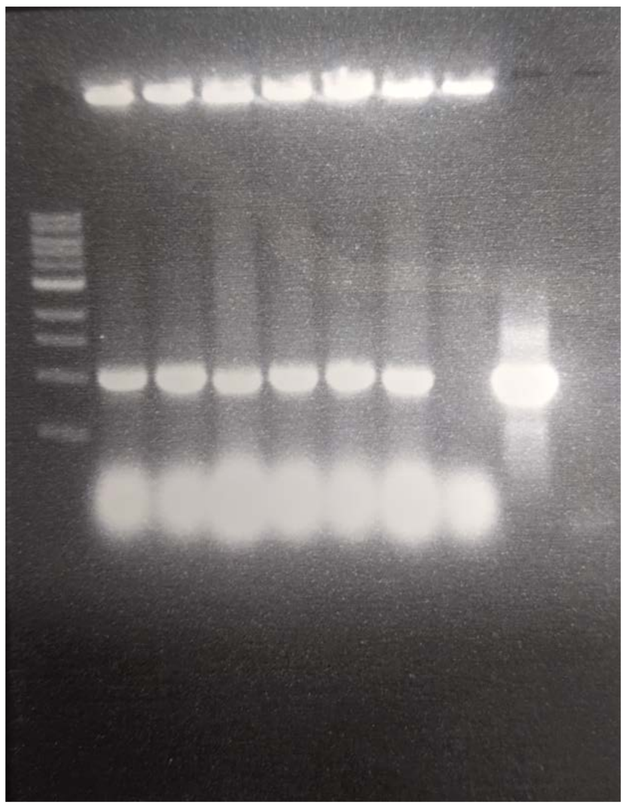


**Figure 1.** Colony PCR of ligation products from transformation in BP5α cells.

## REFERENCES

1. Rice G, et al. (2004). The structure of a thermophilic archaeal virus shows a double- stranded DNA viral capsid type that spans all domains of life. Proc Natl Acad Sci USA 101(20):7716–7720.
2. Samson RY, et al. (2011). Molecular and structural basis of ESCRT-III recruitment to membranes during archaeal cell division. Mol Cell 41(2):186–196.
3. Snyder JC, et al. (2013). Functional interplay between a virus and ESCRT machinery in Archaea. *PNAS 110*(26): 10783-10787.
4. Snyder JC, Young MJ (2011) Potential role of cellular ESCRT proteins in the STIV life cycle. Biochem Soc Trans 39(1):107–110.

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