# Teenage Mutant Spindle-shaped Viruses

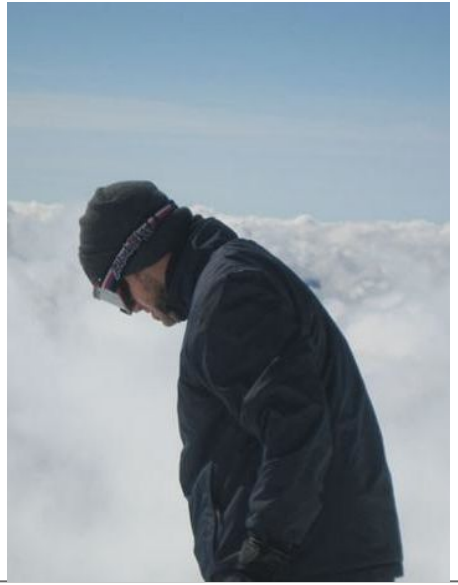
By Rachel Champaigne and Peter Robinson

Two mutant viral genes walk into a bar. One turns to the other and says “How you doing?”

The other one says “Hard to say. I’m having a lot of trouble expressing myself.”

# About us

Rachel Champaigne



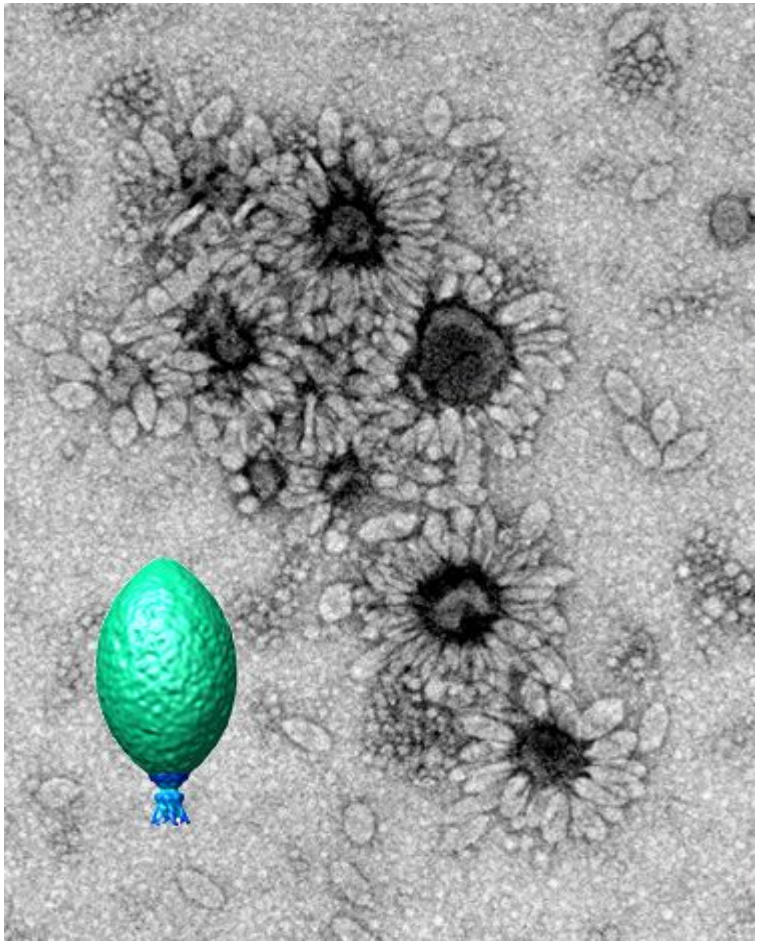
Peter Robinson, Biology Undergrad

# Summary

- Transposons randomly inserted into SSV1 genome
- Protein expression disabled by inserted transposon (structure=function)
- Samples E5 and I6 identified as mutants; F10 non-mutant
- Mutants confirmed via PCR-> gel electrophoresis and sequencing
- E5 transposon determined to be in D335 gene, bp 1015-1016
- I6 transposon determined to be in A291 gene, bp 7898-7899
- Both genes determined to be essential for survival of virus

# Intro and background

- *Sulfolobus solfataricus* is a thermophilic halophilic extremophile archaea found in low pH (~2-3) sulfurous hot springs around the world
- Spindle-shaped virus 1 (family *Fuselloviridae*, genus *Alphafusellovirus*) is a virus that infects *S. solfataricus* cells and integrates its genome into the host genome
- Transposon is a small DNA element to be inserted into a genome
- Plasmid is a small circular DNA element that can exist independent of genome or insert
- Location of insertion can tell us about what the gene at that location does and whether it is critical to survival of the organism

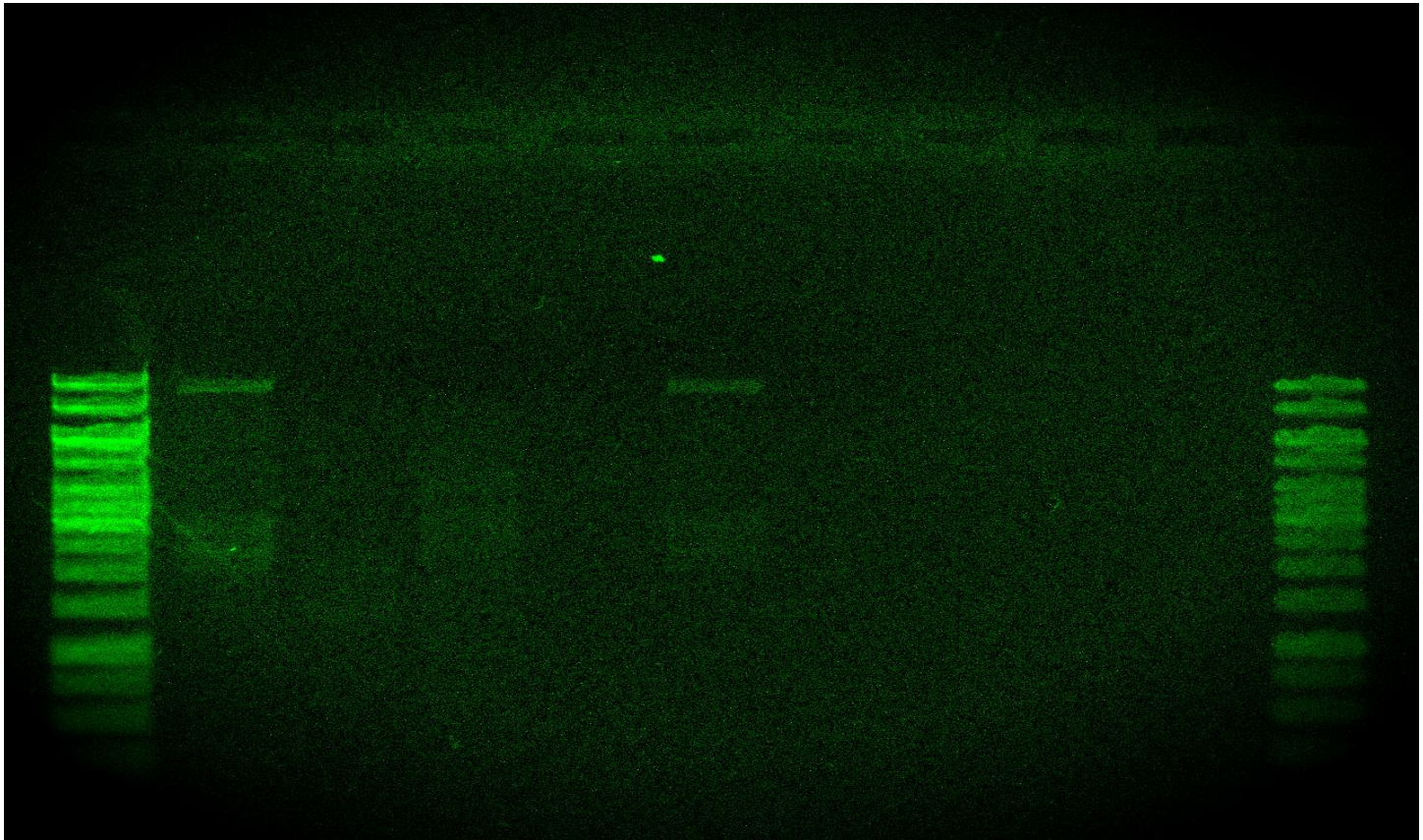


Spindle-shaped Virus 1 and host *S. solfataricus*[1]

# What we did/found

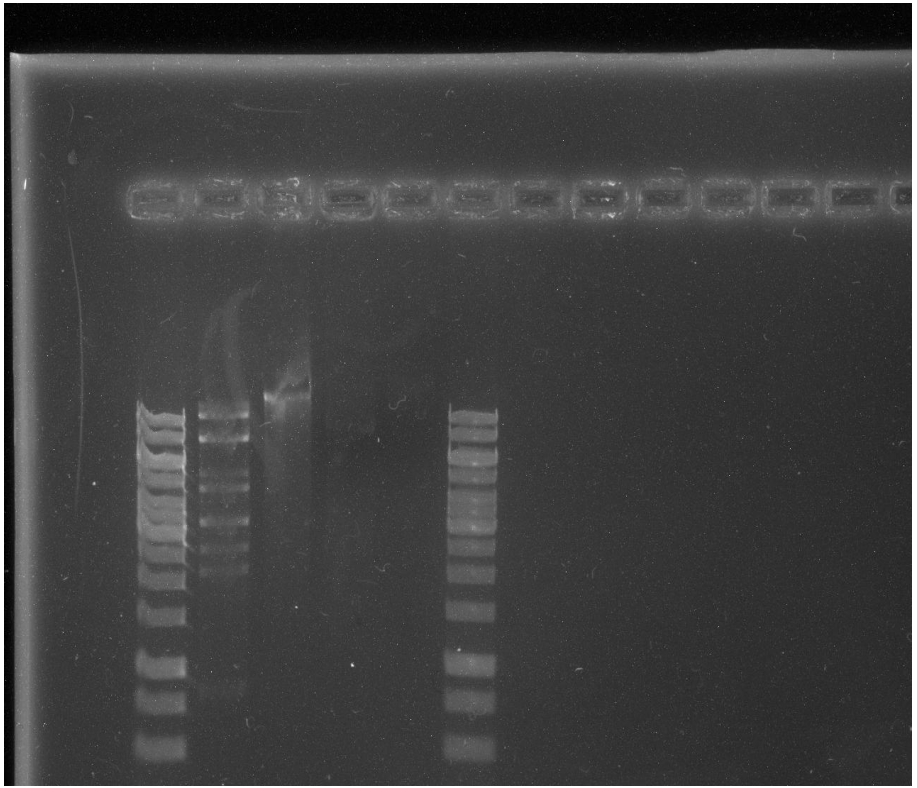
- Received mutants F10, E5, I6 with transposons randomly inserted, *Escherichia coli* as host
- Plasmid DNA isolated and purified
- Restriction digest with EcoRI on viral DNA
- Gel electrophoresis run with F10, E5, I6 samples, compared to wild type
- Additional transposon band of 2 kb found in F10, indicating presence of transposon, not integrated into genome, not a mutant
- E5 and I6 mutants displayed bands not present in wild type; E5 at 9 kb and I6 at 4.5 kb

# What we did/found



# What we did/found

- Determined additional restriction digests to more closely identify location of transposon with NEBcutter
- Restriction digest with EcoRV and SmaI on mutants E5 and I6
- Gel electrophoresis performed
- Mutations were found in the 2.8 kb and 4.1kb bands (E5); and in the 8kb and 4.3kb bands (I6)



# What we did/found

- Electroporation for *S. solfataricus* performed, E5 and I6 introduced to competent cells
- Halo assay performed, no halos present- not functional mutant
- *E. coli* made chemically competent and transformed with mutants, plated in both diluted and concentrated solutions, growth equal for each strain and concentration
- Primers selected for PCR to amplify identified mutant band, 1/2 previous band from double digest per primer set
- *E. coli* lysed via alkaline lysis to release plasmid
- Second halo assay- no halos

# What we did/found

- PCR run to amplify regions dissecting the previous regions identified as containing transposon
- Electrophoresis run
- Mutant bands were at 2.5 kb and 4.5 kb.

# What we did/found

- Mutant DNA was electroporated into *S. sulfolobus*
- BDT prep and sequencing run on amplified PCR product- transposons found to be inserted between bp 1015 and 1016 in mutant E5 and transposons identified to be between bp 7898 and 7899 in mutant I6.
- Interrupted genes were identified to be d335 (integrase) in mutant E5 and a291 in mutant I6
- Halo Assay for transformed *S. sulfolobus* performed. No halos were observed- no functional mutants
- Integrase (d335) and hypothetical protein a291 are important for SSV1's viability

# Discussion- a291

Mutant I6: a291- Hypothetical/uncharacterized protein with no identifiable structural homology to any known protein. A blastx search showed 100% homology with SSV1 gene, as expected, but also found an 80% homology with *Sulfolobus islandicus,* potentially indicating a prophage insertion site, or possible horizontal transfer from SSV1. Oddly enough, there is no homology to SSV2 or 3, viruses that are found in *S. islandicus* when SSV1 is not.

*note- a291 is found on its own transcript, further supporting evidence that it is an important gene for survival. It is an early late gene, and is thought to be involved in capsid formation or as a structural protein

# Discussion- d335

Mutant E5: d335- Integrase gene. *note- In Clore et al, 2006[2], the integrase gene in SSV1 was knocked out and the virus was still determined to be functional. In an attempt to determine the cause of the discrepancy of functionality between Clore and this experiment, some differential factors of the the two studies were identified.

The *S. solfataricus* strain used in Clore et al was P2, isolated from Italy, which has a satellite genetic element (pSSVi) that has been shown to enhance the virulence of SSV1 P2 strain, without being requisite for viability.

Red in rocks at Naples, Italy, a result of *S. solfataricus* colonization.[3.]

# Discussion- S441

The strain of *S. solfataricus* used in our experiment was S441, isolated from Bumpass Hell, Yellowstone National Park, USA. This strain does not have a satellite genetic element. The strain used in Clore's paper[2] was P2, which has pSSVi as a satellite. Wang et al, 2007[4] shows that pSSi has a SSV-like integrase gene in its genome. This information shows one possible reason that the integrase gene is ancillary in the P2 strain, but essential in S441.

# Future Directions

*Check to see if there is an *S. solfataricus* strain susceptible to infection by SSV1 that also has a satellite virus that does not encode an integrase gene. Infect with E5 mutant, if it is not functional, the satellite (pSSVi) in P2 is likely the reason that Clore[2] found limited functionality with integrase knock out. If it is functional, other reasons for the variance, such as regulating RNAs or other regulatory regions can be examined to find what element is necessary to retain the limited functionality found in Clore[2].

# Future Directions

* Examine structure of A291 with x-ray crystallography or molecular modeling, look at areas of homology in *S. islandicus* and SSV1, and areas that are not homologous to identify conserved regions- try to identify popular motifs/domains

Look at regions flanking 80% homologous hypothetical protein in *S. islandicus* to determine if potential viral proteins. If they are, it is likely a prophage, integrated into *S. islandicus*, indicating that SSV1 likely infected this archaea at some time. If the regions flanking the a291 homologous gene are not viral genes, this would indicate horizontal gene transfer at some point; that this protein was at one time also in SSV2 or 3, was lost at some point, but remains in the host genome.

A291 homolog could be knocked out of *S. islandicus* to see if it has reduced functionality, or it could be knocked out and then infected by SSV1 to see if there is a change in functionality

# References

[1]Image courtesy of Morais, M. University of Texas Medical Branch

[2]Clore A. J., Stedman K. M. (2006). The SSV1 viral integrase is not essential. *Virology* 361 103–111 [PubMed]

[3]Image courtesy of Tony Phillips, Science@NASA, 1998

[4]Wang Y, Duan Z, Zhu H, et al. A novel Sulfolobus non-conjugative extrachromosomal genetic element capable of integration into the host genome and spreading in the presence of a fusellovirus. Virology. 2007;363:124–133. [PubMed]

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