#1039A

Experimental and Bioinformatic Analyses of Coevolution of Primate Seminal Proteins and HIV/SIV

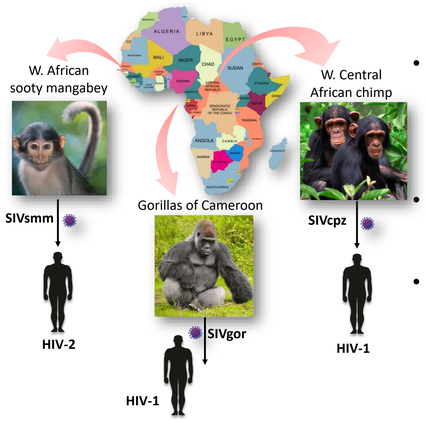
Emine F. Kahveci[1], Feng Hsiao[2], Thomas Mack[3], Ludger Ständker[3], Jan Münch[3], Nadia R. Roan[2], Michael I. Jensen-Seaman[1]

[1]Department of Biological Sciences, Duquesne University, Pittsburgh, PA, USA
[2]Department of Urology, University of California, San Francisco, CA, USA
[3]Ulm University Medical Center, Ulm, Germany

Contact: kahvecie@duq.edu

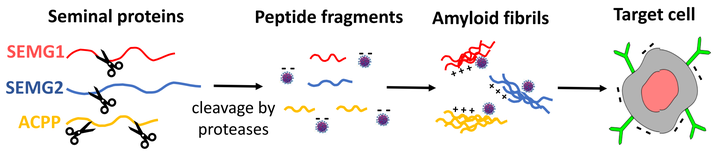
Introduction

- Male reproductive proteins involved in spermatogenesis and fertilization are among the most rapidly evolving proteins in mammals.
- Selective pressures driving the rapid evolution of these proteins are commonly attributed to sexual selection through sperm competition[1,2].
- Defense against sexually transmitted pathogens such as HIV/SIV might also be a contributing factor[2]. (SIV: Simian Immunodeficiency Virus)



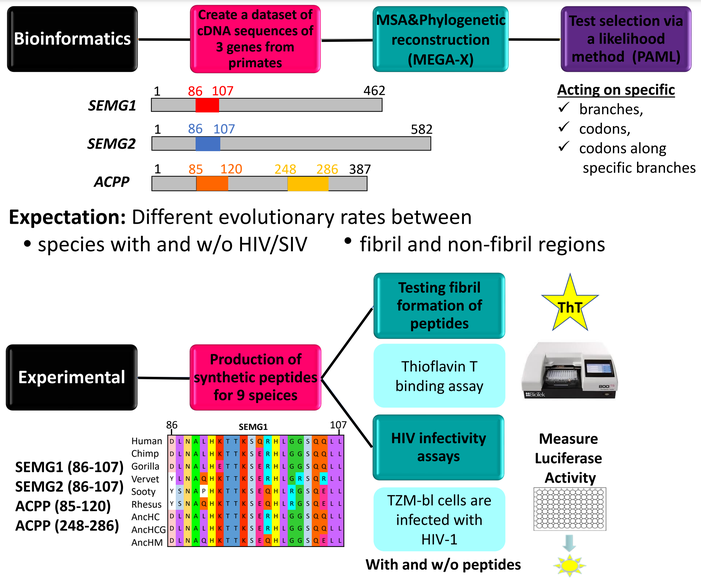
- HIV first entered the human population relatively recently, ~100 years ago through transmission from non-human primates (NHP) in Africa.
- SIV has been infecting ~40 species of NHP for millions of years.
- Therefore, some protective mechanisms might be adapted by species who have evolved with SIV.

- Amyloid fibrils formed from peptides derived from the abundant human seminal proteins semenogelin 1 (SEMG1), semenogelin 2 (SEMG2), and prostatic acid phosphatase (ACPP) dramatically increase the HIV infectivity rate[3,4]. Positively charged fibrils promote the attachment and infection of negatively charged HIV virions on the host cell membrane which is also negatively charged.



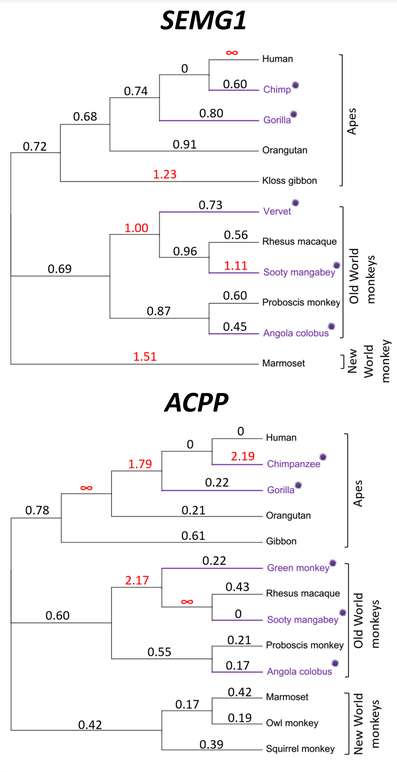
- The **main objective** is to computationally and experimentally test seminal peptide fragments derived from SEMG1, SEMG2, and ACPP in different primates to determine whether these seminal proteins have been evolving in response to HIV/SIV.

Materials and Methods



Expectation: Reduced ability of SEMG1, SEMG2, and ACPP to form HIV-enhancing fibrils and to infect cells in species with a long history of HIV/SIV.

Results



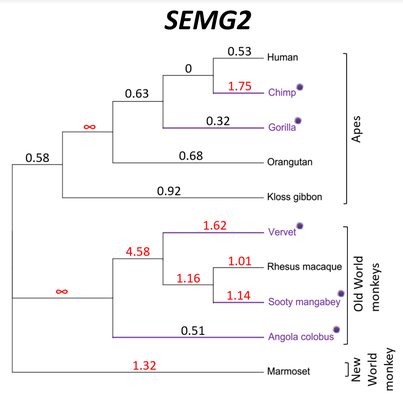


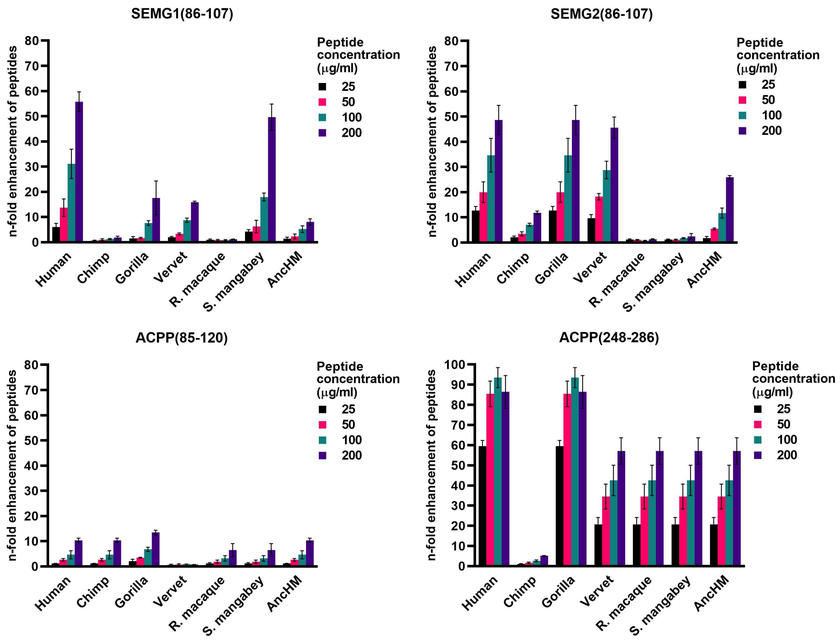
Table. Results of site-specific and branch-site models of *SEMG1, SEMG2,* and *ACPP* genes in PAML

PAML Models	Null Hypothesis	Alternative Hypothesis	*SEMG1*	*SEMG2*	*ACPP*
Site-specific	M0-M1a (nearly neutral)	M0-M2a (positive selection)	117 D, **125 R, 447 R**	73 I, 78 W, **167 W*,** 226 V, 239 H, **250 P, 281 R**,** 310 S, 333 H, **485 T, 554 H*, 565 Q**	129 V
	M7 (Beta)	M8 (Beta&ω)	**78 Q, 117 D*, 125 R*, 208 R, 239 C, 316 S, 424 G, 447 R*, 449 L**	**73 I, 167 W*, 226 V, 250 P, 281 R**, 310 S, 485 T, 554 H*, 565 Q**	109 V, **129 V*,** 135 I, 197 L, 273 R
Branch-site	M2&M0-M2a with ω=1	M2&M0-M2a	None	179 S, 310 S, 321 K, 325 K, 333 H, 364 G	None

Sites with posterior probabilities >0.85 are indicated in regular type, P values>0.90 are in bold.
*Significant P < 0.05 **Significant P<0.01

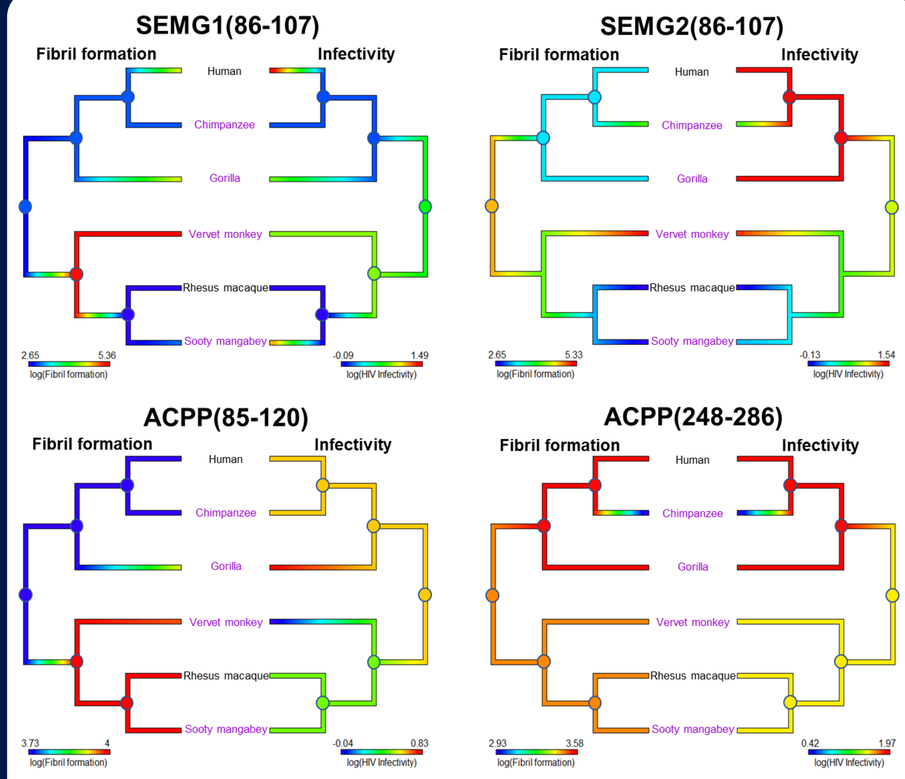
Consensus species trees showing the free-ratio *dN/dS* ratios of ***SEMG1***, ***SEMG2***, and ***ACPP***. The *dN/dS* values greater than one are represented in red. African primates with SIV history are typed in purple. ∞, *dN* is nonzero and *dS* is zero.

- According to the branch-specific model, all three proteins in the species with SIV history have been evolving slightly slower than the species without SIV history.
- None of the positively selected codons identified in the site-specific model are located within HIV-enhancing fibril regions except 109V and 273R in ACPP.



HIV Infectivity Assay Results: The ability of four primate peptides to enhance HIV infection was examined by infecting TZM-bl cells with HIV-1 isolates treated with the indicated concentrations of peptide. N-fold enhancement of peptides was calculated based on the no-peptide control. AncHM refers the hypothetical ancestor of humans, apes, and OWMs.

- Human fibrils strongly enhanced HIV infectivity compared to chimpanzee *in vitro*.
- This strong pattern was not always observed between species w/ and w/o virus history.



ContMap cladogram illustrating log values of fibril formation and HIV infectivity rates in the presence of peptides (100μg/ml) from six primates and some hypothetical ancestors, where warmer colors represent greater activity. African primates with SIV history are shown in purple. Colored circles at the internal nodes represent the hypothetical ancestral states that were empirically tested. An R package "*Phytools*" was used to create these character trees[5].

Discussion and Conclusion

Bioinformatics

- Seminal proteins evolve slightly slower in species with SIV history but this result is not statistically significant.
- Homologs of SEMG1 and SEMG2 fibril regions in human semen do not appear to be evolving significantly differently than the non-fibril regions, however, positively selected codons still might be involved in fibril generation by affecting the cleavage efficiency of peptides.
- HIV-enhancing fibril regions of ACPP evolve slightly faster than the rest of the protein.

Experimental

- Amyloid fibrils from three proteins have decreased infectivity rates in SIV-harboring chimpanzees compared to human fibrils. An exception observed in ACPP(85-120) is due to shared identical peptide sequences between humans and chimpanzees.
- The inclusion of peptides corresponding to hypothetical ancestral sequences allowed us to conclude the direction of change.
- Several seminal proteins might have being evolved in response to sexually transmitted viruses in primates in addition to sexual selection.

Acknowledgements

- This work was funded by NIH (NIGMS): R15 GM123447 (Jensen-Seaman MI).
- Duquesne University, Bayer School of Natural and Environmental Sciences, Department of Biological Sciences Graduate Assistantship

References

1. Jensen-Seaman, M.I. & Li, W.-H. Evolution of the hominoid semenogelin genes, the major proteins of ejaculated semen. *Journal of molecular evolution* **57**, 261-270 (2003).
2. Clark, N.L. & Swanson, W.J. Pervasive adaptive evolution in primate seminal proteins. *PLoS Genetics* **1**, e35 (2005).
3. Münch, J. *et al.* Semen-derived amyloid fibrils drastically enhance HIV infection. *Cell* **131**, 1059-1071 (2007).
4. Roan, N.R. *et al.* Liquefaction of semen generates and later degrades a conserved semenogelin peptide that enhances HIV infection. *Journal of virology*, JVI. 00269-00214 (2014).
5. Revell, L.J. phytools: an R package for phylogenetic comparative biology. *Methods in Ecology and Evolution* **3**, 217-223 (2012).