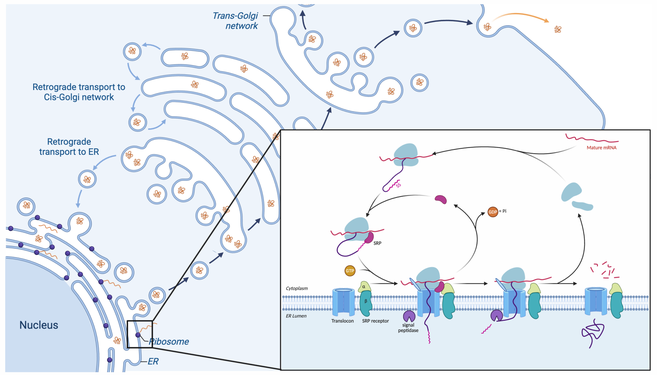
Enhancing Protein Biomanufacturing Using Virus-Derived Signal Peptide Sequences

Vivian Saavedra, Chemical Engineering
Mentor: Dr. Jordan Yaron, Assistant Professor
School for Engineering of Matter, Transport and Energy

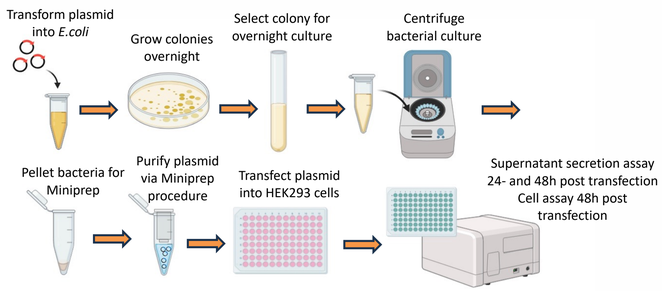
Introduction

- How can we manufacture recombinant biologics, such as therapeutic protein or monoclonal antibodies, more quickly and efficiently, and thereby lower costs?
- Many viruses have evolved to employ efficient and effective immune evasion strategies, in some cases using rapid protein secretion of immune modulating proteins
- In prior work, harnessing naturally evolved, viral solutions to biological problems has been effective at manipulating disease processes, e.g., through the recombinant application of viral immune modulators
- This project investigates a novel aspect of evolved virulence for applications in biomanufacturing by interrogating virus-derived signal peptide sequences for enhanced export of recombinant proteins (NanoLuciferase). The primary goal is to learn how to enhance the secretion of proteins in the biomanufacturing processes for recombinant biologics to increase yields and lower costs.
- To enhance protein secretion, highly potent amino-terminal (N-terminal) signal sequences were investigated that facilitate secretion from mammalian cells.
- Signal sequences are short peptides usually around 16-30 amino acids long that direct proteins to the secretory pathway



Methods

- A panel of 23 virus-derived signal peptide sequences of varying length and origin was developed
- Each plasmid was transformed into endotoxin-free DNA and transiently transfected using PEI-Star reagent into the FDA-approved cell line HEK293
- NanoLuc secretions were evaluated 24 and 48 hours post transfection, and remaining NanoLuc on the cells was evaluated 48 hours post transfection by an *in vitro* luminescence assay



Results

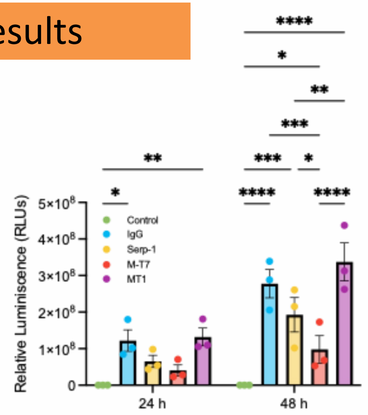


Figure 1

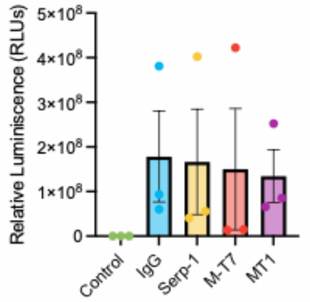


Figure 2

Figure 1: Secreted Luciferase 24h and 48h post transfection. N=3.

Figure 2: Luciferase on cells 48h post transfection. N=3.

Conclusion

- In preliminary studies, MT1 signal peptide sequence showed to secrete the most NanoLuciferase both 24 hours and 48 hours post transfection, followed by IgG, with MT7 showing the least amount of secretion from the four signal peptide sequences selected
- Future work involves further studies and comparisons of protein secretion with different signal peptide sequences

References

Finlay, B.B. and McFadden, G., 2006. Anti-immunology: evasion of the host immune system by bacterial and viral pathogens. Cell, 124(4), pp.767-782.

Yaron, J.R., Zhang, L., Guo, Q., Awo, E.A., Burgin, M., Schutz, L.N., Zhang, N., Kilbourne, J., Daggett-Vondras, J., Lowe, K.M. and Lucas, A.R., 2020. Recombinant myxoma virus-derived immune modulator M-T7 accelerates cutaneous wound healing and improves tissue remodeling. Pharmaceutics, 12(11), p.1003.

Yaron, J.R., Zhang, L., Guo, Q., Burgin, M., Schutz, L.N., Awo, E., Wise, L., Krause, K.L., Ildefonso, C.J., Kwiecien, J.M. and Juby, M., 2020. Deriving immune modulating drugs from viruses—a new class of biologics. Journal of Clinical Medicine, 9(4), p.972.

Acknowledgements

This work was generously supported by Arizona State University through a start-up fund to Prof. Yaron. I gratefully acknowledge my lab mentor, Rushil Kolipaka, and our center director, Prof. Kaushal Rege.

FURI

Ira A. Fulton Schools of Engineering
Arizona State University