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The Pathway Less Taken: Prediction of Fungal Proteins Secreted through Non-classical Pathways

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Introduction

Cycle of Fungal Parasitism

The *Microbotryum* genus of smut fungi is known to parasitize flowering plants by colonizing the plant host and ultimately replacing pollen with fungal spores, which continues the transmission process with the help of pollinators to disperse spores. A hallmark of this genus of fungi is host specialization wherein one fungal species is only capable of infecting one species of plant host. However, there are rare generalists that flout this pattern and one of those generalists, *Microbotryum intermedium*, is the subject of this analysis.

The life cycle of *Microbotryum intermedium* begins when a pollinator transmits spores to an uninfected flower. Meiosis and conjugation take place, and are followed by systemic infection of the plant by the fungus. Once the plant is infected, it begins to look like the flower in image **a**.

Adapted from "Mating System of the Anther Smut Fungus Microbotryum violaceum: Selfing under Heterothallism," by T. Giraud, R. Yockteng, M. López-Villavicencio, G. Refregier, & M. E. Hood, 2008, Eukaryotic cell, 7, p. 769. Copyright 2008 by the American Society of Microbiology.

Methods

Protein Secretion

Secreted proteins are crucial for the interactions between cells and their environments. These proteins play a role in nutrient uptake, cell signaling and hormone release, among many other things. This project's primary objective is to discover predicted secreted proteins and to begin to understand the role that fungal secreted proteins play in host colonization and infection.

During the infection, the fungus synthesizes and releases small secreted proteins (SSPs) into the host plant. SSPs, usually shorter than 250 amino acids, might regulate or alter the gene expression of the host to enhance the fungal colonization.

fungus oomycete

Pathogen structure exploring the apoplast (hyphae)

plant cell

Pathogen structure entering host cell cavity (haustorium)

Host-cell translocated effector proteins

microbial plasma membrane
plant cell wall
plant plasma membrane
extra-haustorial membrane (EHM)

microbial cytoplasm

plant cytoplasm

Image: Petre and Kamoun, 2014

Secretion Pathways

Unconventional trafficking

Leaderless non-vesicular UPS

Pore formation

ABC transporter

Leaderless vesicular UPS

Golgi-bypassing UPS

Conventional trafficking

trans-Golgi

cis-Golgi

Leaderless proteins

Plasma membrane

ER

Nucleus

Kim et al., 2018

Key: Transmembrane proteins (conventional) | ER luminal proteins | Leaderless cytosolic proteins | Transmembrane proteins (unconventional)

Classical

Classically secreted proteins route through the endoplasmic reticulum and Golgi body before being shuttled to their final destination. This is typically via a secretory vesicle that fuses to the cell membrane. All classically secreted proteins have highly conserved sequences called signal peptides, which route proteins to their ultimate destination. These signal peptides are found on the N-terminus of the protein and are generally 16-30 amino acids long.

Nonclassical

Nonclassical routes secrete proteins independently of the endoplasmic reticulum and Golgi body network. These proteins were found during experiments that blocked a classical secretion pathway, but found secreted proteins were still present. Proteins that are released from the cell through alternative routes do not have the N-terminal signal peptides that are conserved in classical routes. Nonclassical secretion is also referred to as leaderless secretion, due to the lack of the N-terminal signal peptide.

SecretomeP 2.0

While other bioinformatics approaches exist for predicting secreted proteins, the goal of this project was to find predicted secreted proteins that use nonclassical secretion pathways to exit the cell. SecretomeP 2.0, a web-based bioinformatics program hosted by the Danish University of Technology, allowed us to accomplish this goal. SecretomeP is a sequence-based method for the prediction of secretory proteins, targeted to the non-classical secretory pathway (Bendtsen and Jentsen et. al., 2004).
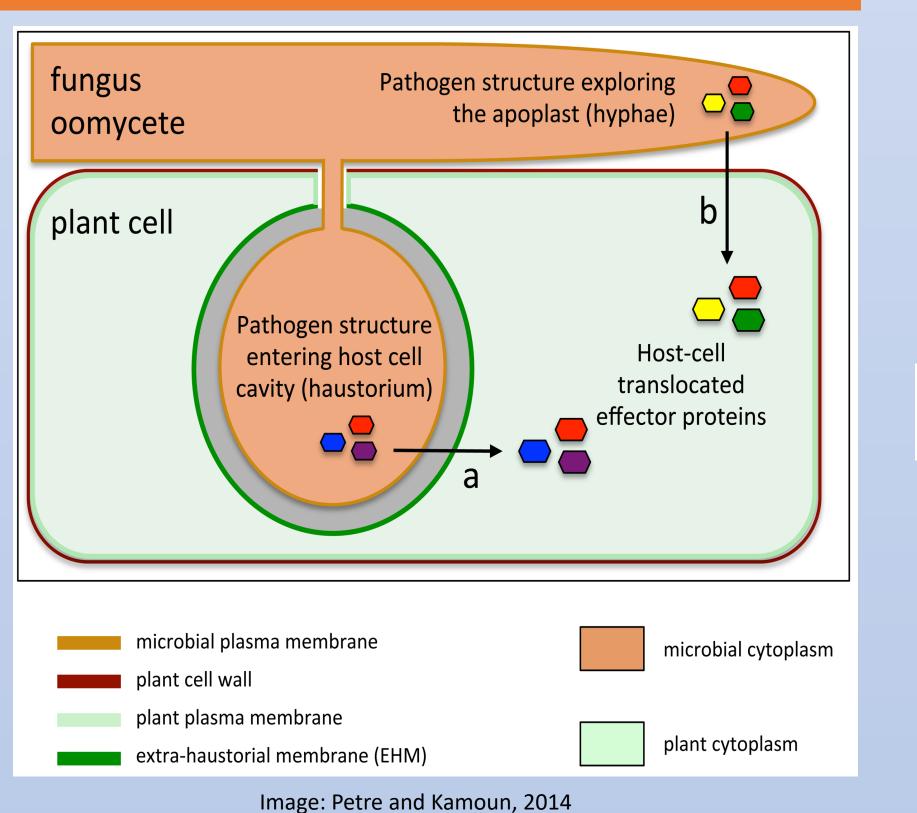
Data Collection and Analysis

The genome of *Microbotryum intermedium* was acquired from JGI Genome Portal. This organism is predicted to have 8,148 genes based on previous gene annotation.

The files containing the protein sequences, sequence IDs, and protein descriptions where they were known, were separated into 100 sequence batched input files for input into the SecretomeP program.

A total of five protein sequences had to be excluded from the analysis because they were shorter than the minimally required 40 amino acids.
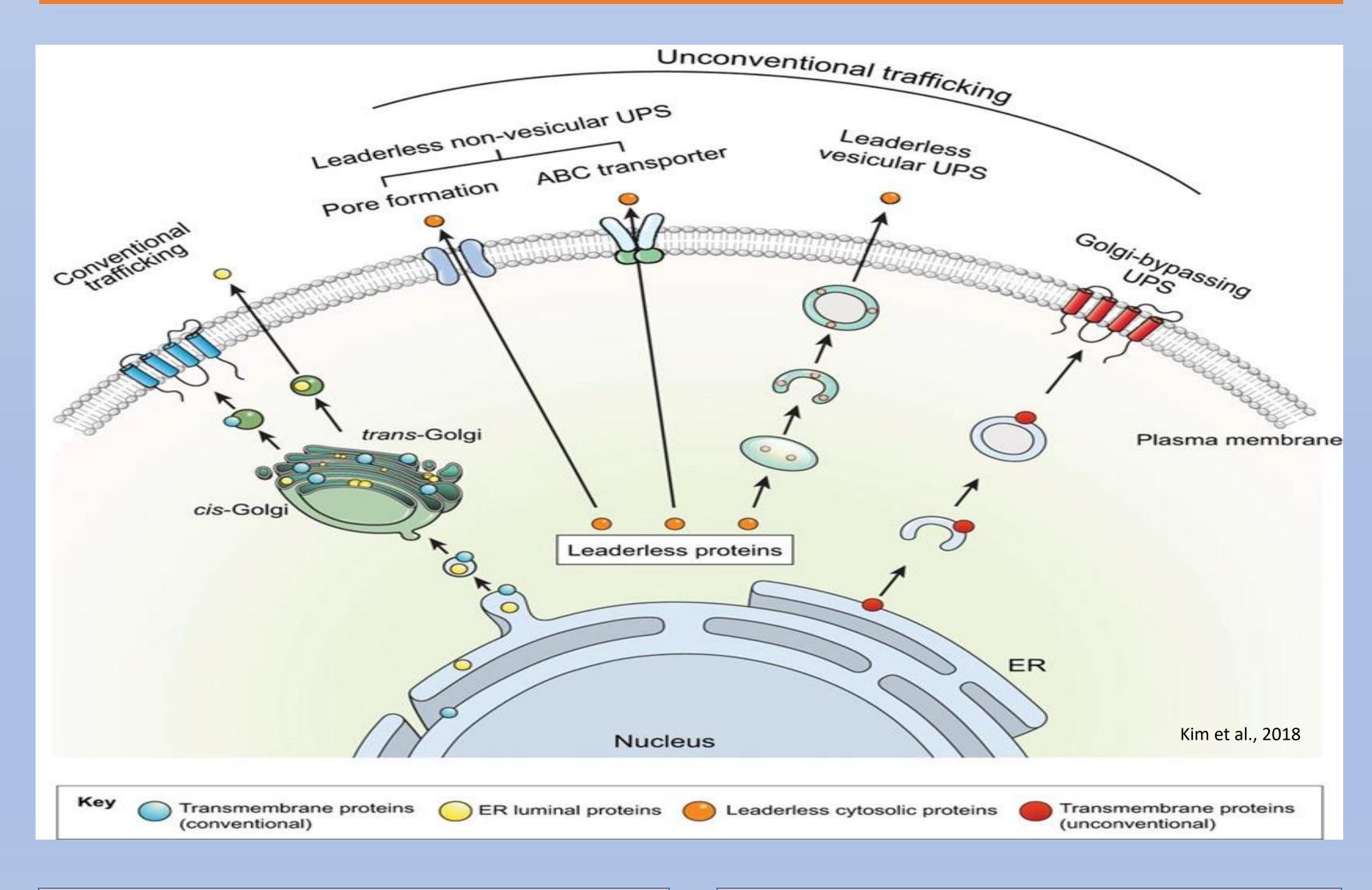
SecretomeP output indicates whether a protein is secreted or not by giving it an NN-score. NN-scores of greater than 0.6 are designated as secreted, but the score does not indicate which route they take out of the cell. To find which pathway the protein takes, the Warning column was checked. If a signal peptide was predicted, that means that it was determined to be a classically secreted protein. In the final data sort, these proteins were removed, because they are not of interest to our current project.

Results and Discussion

Of its 8,148 genes, *Microbotryum intermedium* is predicted to have 2,969 secreted proteins, which includes proteins secreted using classical secretion as well as proteins that use nonclassical secretion pathways. Of the 2,969 total secreted protein subset, 2,679 take a nonclassical pathway, and 300 take a classical pathway.

While this is a larger number of proteins than we anticipated, we speculate that this might be due to a limitation of the program itself. SecretomeP uses a neural network to train its algorithm and the stringency of the calls made depends partly on the dataset that was given when training the neural network. The dataset that was used to train the version of SecretomeP that we used was comprised of known secreted proteins in mammals. Knowing this, we postulate that the number of predicted secreted proteins would be lower if known fungal secreted proteins were used in the training of the algorithm.

Uninfected *Dianthus*

Dianthus anthers infected with *M. intermedium*

Conclusions and Ongoing Projects

- We provide a more complete prediction of proteins secreted by *Microbotryum* during infection
- In future experiments, we will combine these findings with gene expression data to find fungal proteins with significant roles in the infection process; this future goal will be pursued once we have isolated RNAs from infected plants to use for transcriptome analysis
- This analysis will provide additional candidate fungal proteins to test for function and their importance during the infection process

References

Bendtsen, J., et al. *Feature-Based Prediction of Non-Classical and Leaderless Protein Secretion*. 28 Apr. 2004.
Giraud, T., et al. "Mating System of the Anther Smut Fungus Microbotryum Violaceum: Selfing under Heterothallism." *American Society of Microbiology*, 2008, p. 769.
Kim, J. et al. 2018. Unconventional protein secretion – new insights into the pathogenesis and therapeutic targets of human diseases. Journal of Cell Science 2018 131: jcs213686 doi: 10.1242/jcs.213686
Petre, B., and S. Kamoun. "How Do Filamentous Pathogens Deliver Effector Proteins into Plant Cells?" 2014.

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