Wesley Beckham

Thesis Committee: Marcelo Sousa, Halil Aydin, and Harrison Carpenter

The Type Three Secretion System (TTSS) is a syringe-like protein complex that is used by bacteria like *Salmonella* to translocate their infectious proteins (effector proteins) into target cells[1]. The needle of the syringe of the TTSS is narrow, so the proteins need to be unfolded by an unfoldase before they can be translocated[2]. The unfoldase at the base of the TTSS complex cannot resolve highly structured proteins, so if a thermodynamically stable protein such as ubiquitin is fused with the effector proteins, the effector proteins won't be secreted because they can't be unfolded[3]. These prior findings suggested that the effector proteins evolved to be thermodynamically unstable to support secretion through the TTSS[4]. However, other recent studies have shown that effector proteins have similar thermodynamic stabilities to those of normal proteins[5].

We hypothesize that the effector proteins have evolved to be mechanically labile so they could be unfolded by the TTSS complex. The proteins would also maintain thermodynamically stable configurations following secretion to support refolding in the host cell cytoplasm.

Since effector proteins are dependent on the unfoldase, effector protein unfolding at the base of the TTSS complex is likely the rate-limiting step of secretion. Therefore, the thermodynamics of effector protein unfolding should correlate to the rate of secretion through the TTSS. To test this hypothesis, we utilized a reporter system that added a fluorescent reporter tag to an effector protein and a non-effector protein that is homologous to the effector protein. The reporter does not hinder secretion and binds a fluorescent molecule that will be in the solution. Next, the tagged proteins will be transduced into *Salmonella typhimurium* and placed into an infection assay with mammalian cells. Tagged effector proteins will be highly concentrated in the bacterial cells resulting in high fluorescence. Once the *Salmonella* start secreting their proteins into the mammalian cells, the fluorescence will decrease due to the drop of concentration within the bacterial cells. The rate of decrease of fluorescence will correlate with the thermodynamics of effector protein unfolding and control non-effector proteins.

So far I've completed making the plasmids and I am currently working to get the plasmids in *Salmonella typhimurium*.

After this experiment, next steps might include looking at the thermodynamic stabilities of the effector and non-effector proteins being used in this study and comparing them.

Timeline:

1. Make Plasmids that clone for proteins with fluorescent tags
2. Put plasmids into *Salmonella typhimurium*
3. Use *Salmonella typhimurium* in an infection assay and watch fluorescence

References


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