Eukaryotic post-translational modification of bacterial effectors

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Legionella pneumophila, the causative agent of Legionnaire's disease, has only recently become a human pathogen. Its intracellular lifecycle in amoeba, the natural host, has primed the bacteria for invasion into human alveolar macrophages. Co-evolution within amoeba and horizontal gene transfer has helped shape the near 300 effectors produced by *Legionella* that are injected into the host cell by the Dot/Icm type IVB translocation system[1]. A majority of these effectors have eukaryotic-like domains such as: F-box, U-box, Sel-1, ankyrin repeats, leucine-rich repeats, and CaaX motifs, which were likely acquired by horizontal gene transfer[2]. These domains aid in the hijacking of host processes by *L. pneumophila* in order to promote growth and replication. Many injected bacterial effectors are modified by the host through various post-translational modifications, however asparagine hydroxylation modification has never been observed.

Post-translational asparagine hydroxylation of proteins in mammalian cells is mediated by Factor Inhibiting HIF (FIH). FIH is most commonly studied for its role in asparagine hydroxylation which regulates the Hypoxia inducing Factor (HIF), responsible for the transcription of around 100 hypoxia-related genes. FIH recognizes the amino acid sequence Lxxxx(D/E)ϕNϕ[3]. This motif can be found in 11 of the injected effectors of *L. pneumophila*, designated as Hydroxylated Effectors of *Legionella* (HEL). This motif can also be found in other injected bacterial effectors such as, the Outer Protein M (YopM) of *Yersinia pestis*, IpaH4.5 ubiquitin ligase of *Shigella flexeneri*, and an uncharacterized ankyrin protein of *Rickettsia felis.* It is likely that this motif is present in many other bacterial effectors that have yet to be described.

Exploitation of host post-translational modification plays an important role in bacterial pathogenesis by further tuning it with the host, allowing it to manipulate and modulate host functions. *Our hypothesis is that pathogens hijack host FIH in order to hydroxylate effector proteins, making them biologically functional.* To test this hypothesis, three aims are proposed.

Aim 1-Hydroxylation of effector proteins

In order to determine if the HEL proteins of *L. pneumophila*, YopM of *Y. pestis*, and IpaH4.5 of *S. flexeneri* are hydroxylated in human cells, HEK293 cells will be transfected with plasmid containing FLAG-tag fusion proteins. Purified proteins will be analyzed by matrix-assisted laser desorption/ionization (MALDI) Mass Spectrometry (MS), to identify a 16 dalton mass shift in the fragment containing the hydroxylation motif. This will be done in collaboration with Dr. Michael Merchant. To confirm the role of FIH in asparagine hydroxylation, FIH inhibitors and FIH silencing by RNAi will be utilized. We have tested and confirmed hydroxylation in this manner for one of the HEL proteins, AnkH. This gives us reason to believe that others may be hydroxylated as well and supports our reasoning for these studies.

Aim 2- Protein-protein interactions of FIH and effectors

Our preliminary studies have shown colocalization of the host FIH and some of the HEL proteins to the *Legionella* containing vacuole (LCV). Therefore, interaction between FIH and effectors is likely to occur. Due to the transient enzymatic nature of interactions with FIH, Bimolecular Fluorescence Complementation (BiFC) will be used to show the interaction between the proteins of interest and FIH. This system utilizes two plasmids each harboring half of a fluorescent molecule that emits light when brought together by interacting proteins fused to either half. If fluorescence can be detected by confocal microscopy in cells transfected with two plasmids, containing the N-terminal portion of the fluorescent molecule fused to either FIH or effector protein and the C-terminal portion of the fluorescent molecule fused to an effector protein or FIH, then interaction between of the two proteins can be suggested.

Aim 3- Role of asparagine hydroxylation motif in the biological function of effectors

Generating point-mutations in the asparagine of the hydroxylation motif for each protein will elucidate how hydroxylation of this residue is important to the function of the protein. This will be in comparison to the knock-out mutant, lacking the gene, which will also be generated. These mutants will be used in functional studies in a variety of species and cells such as human derived macrophages, mice, and amoebae. Because *L. pneumophila* has a plethora of hosts, it is possible that a mutant has an effect in one species or type of cell but not another. This will also be done with *Y. pestis* YopM mutant in human macrophage cell line, in collaboration with *Yersinia* researcher Dr. Matthew Lawrenz.

Hydroxylation of bacterial proteins has never been shown before. This post-translational modification could be the key to more refined modulation and regulation of the host. This motif seems to be abundant in human pathogens and has implication in convergent evolution of bacterial effectors to better survive in its mammalian host. Not only will this educate us on bacterial host interactions but also provide more insight on FIH, as little is known about the nature of FIH hydroxylation outside of its role in hypoxia.

Broader Impacts: These studies would lead into knowledge about effector proteins in the study with unknown function. Ultimately better understanding bacterial effectors and their role in the host could result in potential targets for novel treatments. My research will provide new insights into bacterial protein post-translational modification, and be added to publically accessible databases designed to predict protein structure and function. This will allow others to use this information to elucidate novel functions or regulatory mechanism for proteins in other species.

References

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