Ehrlichia chaffeensis TRP 32 trafficks to the host nucleus in a phosphorylation dependent manner and binds host DNA via a tandem repeat domain

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Ehrlichia chaffeensis is an obligately intracellular bacterium that causes human monocytotropic ehrlichiosis (HME), an emerging tick-borne zoonosis. A group of tandem repeat protein (TRP) effectors that have multiple functions and interactions with the host cell have been identified and associated with E. chaffeensis survival. We previously determined that TRP120 is a nucleomodulin that directly binds a GC-rich DNA motif via a novel tandem repeat (TR) DNA binding domain and likely modulates host gene expression levels. Notably, another effector, TRP32, is highly upregulated during infection of human monocytes and predominately localizes to the nucleus when ectopically expressed, but is also associated with the ehrlichial inclusion in infected cells. The purpose of this project is to determine if TRP32 is a nucleomodulin and to define the mechanisms involved in its nuclear translocation. First, to examine DNA binding, an electromobility shift assay (EMSA) was performed using full-length TRP32 and the TR domain alone. Both the full-length construct and the TR domain alone were shown to bind to human genomic DNA. To study the requirements for nuclear localization, GFP-tagged constructs were created and transfected into HeLa cells and IFA was used to determine subcellular localization. We found that the C-terminal portion of the protein was required for nuclear localization, which could be inhibited by treatment with genistein, a tyrosine kinase inhibitor. In silico prediction identified two tyrosine motifs (Y168 and Y179) within the C-terminus as potential targets for phosphorylation. To determine if these residues are important for nuclear localization, Y168F and Y179F point mutants were constructed. When analyzed by IFA, the Y179F mutant showed decreased nuclear localization compared to wild-type TRP32. In conclusion, TRP32 appears to undergo phosphorylation dependent nuclear localization and interacts with host genomic DNA via its tandem repeat domain.