

Tick-borne flaviviruses, we analyzed the TBFV polyprotein for known binding motifs of antiviral proteins and found four putative tumor-necrosis factor receptor-associated factor 6 (TRAF6) binding motifs (TBM) within the viral nonstructural 3 protein (NS3). TRAF6 is an E3 ubiquitin ligase that is associated with antiviral innate immune signaling. NS3 plays essential enzymatic roles in cleaving the viral polyprotein and replication of the viral genome, making it an ideal antiviral target. Here we report that NS3 from Langat virus (LGTV), a prototypical TBFV, interacted with TRAF6 during infection, as revealed by co-precipitation assay and confocal microscopy. To determine whether the NS3-TRAF6 interaction inhibits TBFV replication, we infected TRAF6<sup>-/-</sup> fibroblasts with LGTV. Surprisingly, we found the replication of LGTV in TRAF6<sup>-/-</sup> fibroblasts was decreased up to 14.2-fold when compared to wild-type cells. Assessment of the highly virulent BSL-4 TBFVs, tick-borne encephalitis virus and Kyasanur Forest disease virus, also found that TRAF6 is needed for optimal replication. The proviral role of TRAF6 appears to be specific to TBFVs, as replication of the mosquito-borne flaviviruses, West Nile virus and Kunjin virus, was enhanced, rather than restricted, in the absence of TRAF6. Site-directed mutagenesis revealed that an E117A mutation located in the second TBM of NS3 resulted in disruption of TRAF6 binding and 82.5% reduction of protease activity. Furthermore, replication of LGTV with an E117A mutation in NS3 was attenuated up to 17.5-fold *in vitro* compared to wild-type virus. Taken together, these studies reveal new insights into how flaviviruses exploit innate immune system signaling for the purpose of viral replication and identify a potential target for therapeutic design.



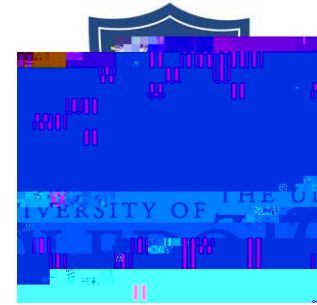
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DISSERTATION  
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*The Role of Tumor Necrosis Factor  
Receptor-Associated Factor 6  
(TRAF6) in Tick-Borne Flavivirus  
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The proviral role of TRAF6 during fla-  
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