CONSIDER FOR TALK

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Lehigh University

Bethlehem PA

Corresponding Faculty Member: Vassie Ware (vcw0@lehigh.edu)



Vassie C Ware

Identification of a Role for Mycobacteriophage Butters-encoded Proteins in a Host Defense Mechanism Against Viral Attack

Catherine M Mageeney, Marta Dies Miracle, Javier Buceta, Vassie C Ware

Our interest in Mycobacteriophage Butters (an N cluster phage) originally stemmed from the discovery that this phage has one of the smallest known annotated genomes (41,491bp with 66 genes, including 4 genes [*gp30*, *gp31*, *gp33*, *gp34*] with no known mycobacteriophage family members [called orphams]). Genome comparisons with other N cluster phages show extensive nucleotide conservation in structural assembly genes in the left arm and considerable divergence in nucleotide sequence and gene content in the central region of the genome, referred to as the “variable region”. Considering the large variation in genome size among mycobacteriophages (41,077-164,602bp), Butters was not only an ideal candidate to explore questions about minimum size requirements for genome packaging, but it was also ideal to determine if its orphams (located in the variable region) are required for a productive lytic or lysogenic life cycle. Recently, a novel mechanism of prophage-mediated immunity against viral attack has been reported for N cluster mycobacteriophages (Dedrick *et al*., 2017). This novel mechanism appears dependent upon genes within the variable region and enables N cluster lysogens to defend against attack from a diverse group of heterotypic mycobacteriophages. We have explored the role of Butters *gp30* and *gp31* (both co-expressed in the Butters lysogen) and report on their contributions to the Butters defense system against viral attack. Initially, computational analyses predict that Butters *gp31* encodes a 4-pass transmembrane protein and that *gp30* encodes a protein with no membrane domains. We tested the computational hypothesis by expressing a C terminal tetracysteine-tagged GP31 and control ORFs within *E. coli* and imaged tagged proteins using fluorescence microscopy. Data show that Butters GP31 resides within the *E. coli* membrane coincident with a membrane marker. GP30 is cytoplasmic within *E. coli* with no membrane overlap. When tagged GP30 is co-expressed with untagged GP31, GP30 appears to be sequestered at the membrane, showing that the presence of GP31 alters GP30 localization. We hypothesize that GP30 and GP31 may therefore interact at the membrane. Immunity experiments using *Mycobacterium smegmatis* strains that express *gp30* or *gp31* alone, or both genes, show that defense comparable to that mounted in the Butters lysogen can be recapitulated only in the *M. smegmatis* strain that expresses *gp30* alone. Taking the imaging and immunity data together, we propose that GP30 is instrumental in conferring defense against specific viral attack only in its cytoplasmic state. What factors regulate a proposed release of GP30 from the membrane (mediated through GP31) are unknown, but other genes (expressed in the lysogen) from the variable region may contribute to regulating this phenomenon. A model for Butters defense against viral attack involving *gp30* and *gp31* will be presented.