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Exploring Cluster N Mycobacterium Prophage Genes Involved in Host Defenses

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Cluster N *Mycobacterium* prophages can protect their host against heterotypic viral attacks by expressing proteins encoded by genes located primarily within the central variable region (CVR) of their genome. We explore the involvement of CVR genes of mycobacteriophage Smurph and a mycobacteriophage Butters reverse gene (gene *57r*; located outside the Butters CVR) in mediating defense in *Mycobacterium smegmatis* mc2155 (*M. smeg*) against heterotypic viral attack. Using bioinformatics analyses, gene deletion strategies using Bacteriophage Recombineering of Electroporated DNA (BRED) technology, and phage plating efficiency assays, we show that deletion of the Smurph CVR (genes *31-35*) abolishes prophage defense against heterotypic infection by PurpleHaze (subcluster A3) but maintains defenses against Bxz2 (subcluster A3) and TM4 (subcluster K2). Interestingly, although the Smurph CVR shares 100% nucleotide identity with the CVR of MichelleMyBelle (MMB), deletion of the MMB CVR gene pair *29* and *30* (homologous to Smurph genes *31* and *32*) is sufficient to abolish defense against infections by Bxz2 and TM4 on *M. smeg*, as reported by Dedrick *et al*. (2017). Differences in immunity patterns for Smurph and MMB suggest that expression of additional Smurph prophage genes may be required to account for immunity pattern differences with MMB. Studies exploring the role of Smurph genes *31-32* and genes *33-35* in host defense against PurpleHaze, Island3 (subcluster I1), Bxz2, and TM4 are in progress. Further, in a Butters lysogen, reverse gene *57r* is necessary to block an Island3 infection, which appears to occur at a late step in the infection cycle. We are investigating the stage at which Island3 infection is blocked. Using *in vitro* strategies to lyse Butters lysogen cells challenged with Island3, we are exploring the hypothesis that gp57r may interfere with the lysis step in an Island3 infection of a Butters lysogen. For both Smurph and Butters prophage-mediated defense investigations, studies are currently ongoing by Advanced Phage Research course students.