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Genetic Analysis of Phages Lacking Puataive 1TMD and 2TMD Lysis Genes Reveals that Both are Required for Efficient Host Lysis and that the 1TMD Protein Regulates the Lysis Event

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Holins are small transmembrane domain (TMD) proteins that serve to control the timing of bacterial lysis after phage infection and for canonical holins, generate lesions in the membrane for the exit of endolysins. Bioinformatic analysis of ~2,500 phages that infect M. smegmatis, shows that the lysis cassette is localized downstream of the minor tails when the genome is <100,000bp and contains at least two genes that encode TMD proteins with the most distal gene usually having a single TMD. The exceptions to this are cluster A phages that have a lysis cassette with endolysins and one TMD gene in the 3’ arm and cluster B and W phages that have a 3-4 TMD gene operon in the 3’ arm and the endolysins downstream of the minor tails. To evaluate the role of the TMD genes in the lysis pathway, they were deleted in the F1 cluster phages Girr and NormanBulbieJr (NBJ). Both Girr and NBJ have a canonical lysis cassette that contains lysin A, lysin B, a 2TMD gene (Girr 34; NBJ 32) and a distal 1TMD gene (Girr 35; NBJ 33). Deletion of Girr 34 or NBJ 32 are not lethal and present with a modest ~40% reduction in plaque size, 30-45 min greater lag time to lysis, and a 10-20% reduced burst size compared to WT phage. The plaque size phenotype can be complimented by infecting M. smeg cells induced to express Girr gp34 or D29 gp11. In stark contrast, deletion of the 1TM gene 35 (Girr) or 33 (NBJ) results in a severe lysis defect manifest by >70% reduced plaque size (>85% reduced plaque volume), limited liquid lysis and a 90% reduced burst size. The exogenous expression of NBJ gp34 or Girr 35 is lethal, but the small plaque phenotype can be complimented by the 1TMD gp32 from phage Waterfoul or 1TMD gp30 from phage D29. Since the plaques in the GirrD35 or NBJD33 mutants are reduced by >70%, repeated infection and plating results in recovery of lysis escape mutants (LEM) that manifest with a wild type plaque size. The LEM phages lyse the bacteria 30-45 minutes earlier than wild type phage in liquid lysis assays and one-step growth curves and have burst sizes reduced by 70% due to the early lysis time. Full genome sequencing showed that the point mutations in the LEM mutants mapped to Girr 34 or NBJ 33. We hypothesize that the point mutations create a 2TMD protein that is unregulated and thus, is able to lyse the bacteria prematurely. Similar LEM mutants were also isolated from D29D30 phage that map to the 2TMD gene 11. Collectively, these finding support the hypothesis that both TMD proteins are required for efficient lysis of M. smegmatis and that the 2TMD protein is inactive (antiholin function?) and is regulated to an active form by the presence of the 1TMD. The presence of both active and inactive TMD proteins in the lysis pathway is consistent with most phages that have been genetically studied. We thank the Hatfull lab for phages and for sequencing mutant phages, HHMI for support of this project and Danielle Heller for helpful discussions and NBJ phage constructs.