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2025 SEA Faculty Meeting Abstract

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An essential A1 immunity repressor gene in the F2 mycobacteriophage Soul22

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Over 2600 mycobacteriophages have been sequenced and their genomes analyzed. Both lytic and temperate phages are represented in the collection. Lytic infections produce and release new phage whereas temperate phages can enter a “dormant prophage state” in a host cell (lysogen), with no new phage produced unless stimulated into lytic growth. The host cell lysogen can typically block infections by phages similar to the resident prophage (superinfection immunity) through expression of a phage-encoded immunity repressor (IR) gene. IR proteins bind to specific sequences located in the phage genome and block expression of lytic-promoting genes. Soul22 is a temperate F2-cluster mycobacteriophage that unexpectedly contains two IR genes: the Cluster F-specific Soul22\_49, and Soul22\_44 which is 93% identical to immunity repressor gp69 of A1 mycobacteriophage Bxb1. As predicted, Soul22 lysogen is immune to both A1 and F2 phage infections. But surprisingly, Soul22\_44 is essential for Soul22 reproduction in wild-type *M. smegmatis* (MC2155) host cells; it can be propagated in *M. smegmatis* expressing Soul22 gp44 from a plasmid. We reasoned that Soul22 gp44 may be needed to repress the expression of a ‘toxic’ gene(s) of either the phage or the host. Just one perfect match (13/13) to the Bxb1 consensus binding site (A1IR-BS, CTTGACACGTAAC) is present in the Soul22 lysogen and that is in the reverse strand of the phage genome at coordinates 31971-31959, immediately upstream of the adjacent Soul22\_45 gene (31934 – 31599). A plasmid carrying Soul22\_45 but lacking the adjacent A1IR-BS, but not one containing the A1IR-BS, was stably transformed into *M. smegmatis*, and in another surprise, was not toxic to cells. A plasmid carrying Soul22\_45 with the adjacent A1IR-BS was stably transformed into the lysogen, *M. smegmatis*(Soul22), presumably due to the presence of the A1IR gene product of Soul22\_44. Finally, deletion of genes 44 and 45, but not the A1IR-BS sequence, in phage Soul22 (Soul22\_∆44-45) was successful and lysogens were sensitive to A1 phage infections. These findings point to a surprising interaction between Soul22\_44, Soul22\_45, and the adjacent A1IR-BS in this F2 mycobacteriophage genome.