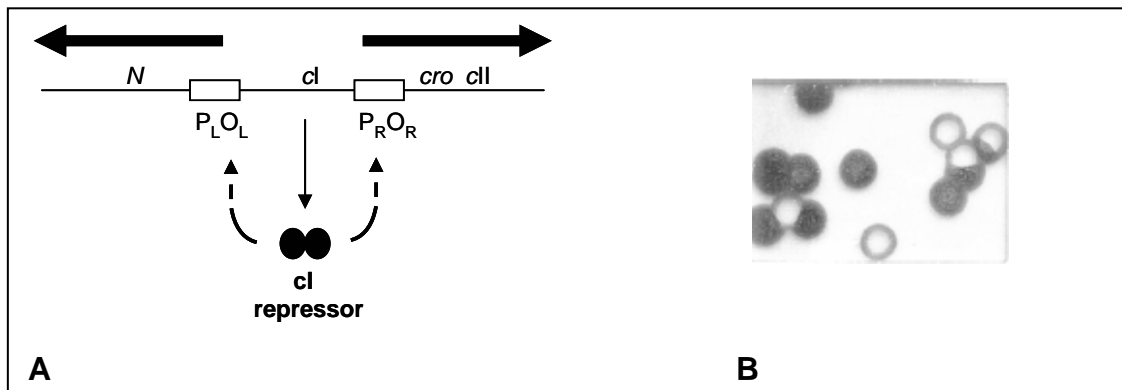


Scenario: Phage Wars
Identification of a Bacteriophage 80 α Lysogenic Exclusion Gene That Targets Phage ϕ 11

Background:

1. **Phage immunity.** Temperate bacteriophages are capable of establishing a stable, “inert” state within infected cells that allows replication and transmission of the phage genome along with that of the bacterial chromosome. This so-called prophage state is maintained by a phage-encoded immunity repressor that prevents expression of the phage lytic genes. Bacterial cells carrying prophages are called lysogens. The immunity repressor binds to specific DNA sequences, called operators, to repress phage transcription. In a lysogen, the repressor gene is one of the few phage genes that is actually expressed. As a consequence of repressor expression, infection of a lysogen by an incoming phage that carries the same operator regions will also be blocked due to repressor binding to the operators on the incoming phage DNA. Hence, bacteria lysogenic for a particular phage are said to be immune to superinfection by the same phage. It is this property that actually gives rise to the “turbid” plaque morphology that is characteristic of temperate phages; the plaques appear cloudy rather than clear because of phage-resistant lysogenic bacteria growing up within the plaques.

Figure 1.(A) Schematic of a typical phage immunity region (this example is from phage λ) showing the leftward and rightward transcripts, initiating from promoters P_L and P_R , respectively. Repressor binds to O_L and O_R , the leftward and rightward operators, to block this transcription. (B) Examples of clear and turbid phage plaques.



2. **Lysogenic conversion**

In addition to immunity to superinfection, acquisition of a prophage may induce other changes in the phenotype of the bacterial lysogen. This phenomenon is called “lysogenic conversion.” In some cases, this is because integration of the prophage interrupts a host gene and disrupts its function. More commonly, however, it is because the phage carries some additional genes, that it picked up via recombination or horizontal transfer, which are nonessential for phage growth and are expressed from the prophage. Sometimes these genes are toxins or other virulence factors or provide the lysogenic bacteria with some additional “fitness.”

Some characterized lysogenic conversion functions are listed in the table below.

Examples of Lysogenic Conversion Genes
Toxins
diphtheria toxin
botulinum neurotoxin
erythrogenic toxin A (Scarlet fever)
Shiga toxins
cholera toxin
Pore-forming cytotoxins
Staphylokinase
Effectors of signal transduction
G protein-like virulence factors
O-antigen serotype conversion
Complement resistance
Antibiotic resistance
Restriction/modification systems
UV induction
Phage exclusion
Reverse transcriptase

One important class of genes that enhance survival of a bacterial lysogen are those that render the cell resistant to infection by other, unrelated bacteriophages. This is mechanistically different than the immunity to superinfection by related phages discussed above, and may target phage adsorption, replication, or other functions. The ability of a prophage to block infection by an unrelated phage is called “lysogenic exclusion.”

3. Temperate phages of *Staphylococcus aureus*

A large number of temperate ds DNA phages that infect *S. aureus* have been characterized, and all sequenced *S. aureus* strains carry one or more prophages. All of these phages are related to each other; they share a similar size and overall genome organization. One of the most striking characteristics of these phages, as has been seen in other groups of phages as well, is the mosaic nature of their genomic structure. Groups of genes can assort among these phages by recombination, giving them enormous genetic diversity. Shown below is a comparison of several of these phages, with regions of sequence conservation between the different genomes highlighted.

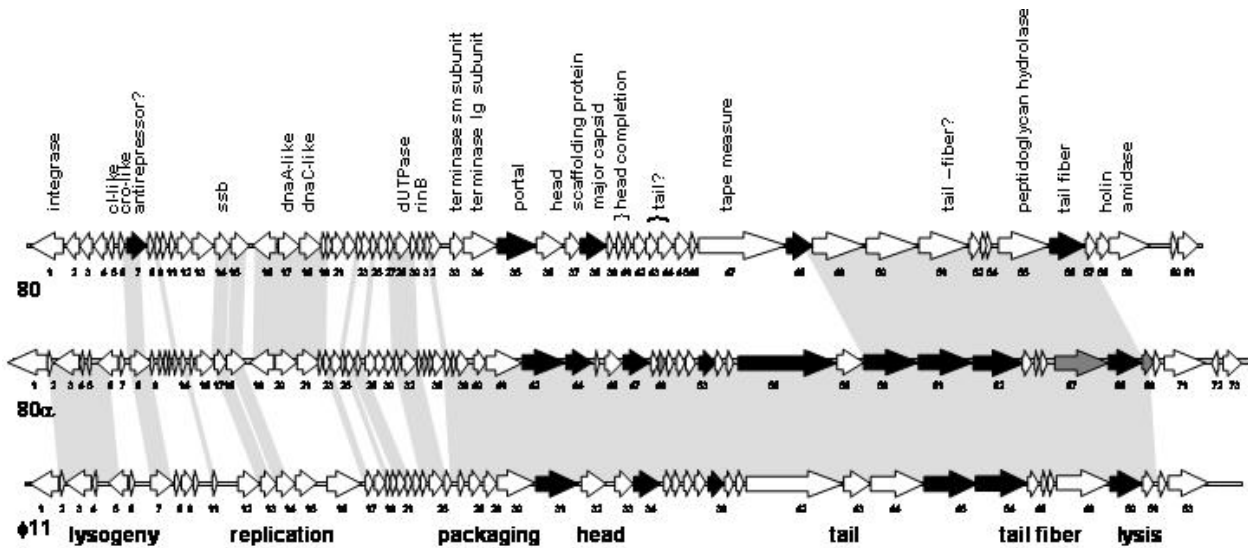


Figure 2. Comparison of the genetic maps of staphylococcal phages 80, 80 α and ϕ 11. Arrows indicate extent and direction of predicted open reading frames; black arrows indicate those genes whose products have been identified in virions thus far by mass spec analysis of major protein bands excised from SDS-PAGE and grey arrows designate low abundance virion proteins identified by mass spec analysis of peptides derived from whole virions. Gene functions inferred from similarities to known genes are indicated and the organization of functions is shown below the map. Shading denotes regions of homology.

Phages 80 α and ϕ 11 share significant sequence similarity, especially in the morphogenetic gene cluster (the genes encoding the proteins that make up the phage particle). These two phages are “heteroimmune”, meaning that their immunity repressors do not recognize the same operator sequences. There is no homology between the N-terminal parts of their repressors, which is the DNA binding domain, and the cloned ϕ 11 repressor does not block 80 α plating or vice versa. However, although 80 α will plate on a strain lysogenic for ϕ 11, the converse is not true. ϕ 11 does not plate on an 80 α lysogen. This indicates that 80 α encodes a lysogenic exclusion gene that blocks the lytic growth of ϕ 11.

The problem – identify potential candidates for the lysogenic exclusion gene.

In order to identify the lysogenic exclusion function, the goal of this exercise is to carry out a comparison of the 80 α and ϕ 11 genomes and develop a prioritized list of candidate genes that should be tested. A lysogenic exclusion gene (1) should be present in the 80 α genome and absent from the ϕ 11 genome, (2) would be expected not to encode a function essential to the lytic growth of the phage, and (3) would be expected to be expressed from a prophage (i.e. not controlled directly by the phage immunity repressor or in a cluster of genes expressed only during lytic growth). This will require making some educated guesses about gene function, based on the existing genome annotations and on similarities of some of these gene products to other known proteins, and also some educated guesses about clusters of co-expressed genes, based on phage biology and predicted promoters.

The phage 80 α and ϕ 11 genomes can be found in the NCBI database under accession numbers NC_009526 and NC_004615, respectively.