Superinfection Immunity

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Glossary

cI The phage lambda (λ) repressor; it is necessary for maintaining lysogeny and acts by inhibiting, that is, repressing the transcription of phage genes required for the display of lytic cycles.

Homoimmunity Describes the relationship between two temperate phages whose lysogens are immune to superinfection by the other's phage particles, in contrast with heteroimmunity where two temperate phages are not mutually immune.

Immunity (or superinfection immunity) Display of homoimmunity against superinfecting phage particles.

Lysogen A bacterium harboring a phage genome, as a prophage, that is capable of a productive infection given induction.

Lysogenic cycle (or infection) An infection by a temperate phage, that is associated with the formation of a bacterial lysogen and continued maintenance of a prophage.

Productive infection A phage infection that results in virion production and either lytic or chronic virion release; most productive infections are lytic, resulting in host lysis.

Prophage An intracellular phage genome stably associated with a bacterium during a lysogenic infection; often but not always found integrated into the host chromosome.

Temperate phage A phage capable of displaying lysogenic as well as productive infections; most temperate phages have lytic rather than chronic productive infections and, following phage adsorption, productive rather than lysogenic infections often predominate.

Secondary adsorption Phage adsorption of an already phage-infected bacterium; depending upon circumstances this may or may not result in superinfection.

Superinfection Entrance by a phage into the cytoplasm of an already phage-infected bacterium; depending on circumstances this may or may not lead to lysogenic or lytic infections.

Introduction

Phage life cycles can be distinguished into two main types, productive versus lysogenic. Unlike productive infections, in lysogeny phages produce no virions and instead are maintained as intracellular prophages that are able to block infections by similar phages. This resistance occurs via the same mechanism that stabilizes existing lysogenic cycles: transcriptional repression of those phage genes required for the productive infection. The inhibitory impact of prophage-produced transcriptional repressor proteins on subsequently infecting phages can be described as an immunity to superinfection, superinfection immunity, or simply immunity. Superinfection immunity has been most thoroughly characterized in phage λ .

Lysogeny and the *cI* Repressor

During lysogeny, a prophage must prevent the transcription of genes whose expression can result in the induction of productive infections. In phage λ , the protein responsible for this transcriptional repression is encoded by the *cI* gene and the product of the *cI* gene, the CI protein, is commonly described as the λ repressor.

Establishment of lysogeny involves what is known as a lysis–lysogeny decision, which occurs 10–15 min after the start of infection with phage λ under standard laboratory conditions. Lysogenic cycles result when levels of λ CII protein are high, whereas lytic cycles occur when levels of CII protein are low. CII protein, in particular, stimulates the expression of the *cI* gene by activating a promoter, *P_{RE}*, that

controls *cI* gene transcription. As long as sufficient CI protein is present, lysogeny will be both established and maintained. Once lysogeny has been established, CI protein stimulates the production of additional CI protein; in this case by acting on an additional *cI* promoter, *P_{RM}*. By also inhibiting *cI* expression at higher CI concentrations, relatively constant levels of CI protein are maintained within λ lysogens under normal growth conditions.

Superinfection Immunity and Its Bypass

The CI repressor blocks not only prophage transcription but also homoimmune phages that happen to infect the same cell. The result is a blocking of infection progression, that is, a display of superinfection immunity. Wild-type temperate phages that fail to respond in this manner are described instead as heteroimmune. Heteroimmune phages are not only capable of productively infecting established lysogens but are also able to lysogenically infect those same cells, resulting in polylysogeny. Polylysogeny may also result given superinfection by homoimmune phages if integrase genes, required for prophage establishment, are not controlled by the resident prophage's repressor.

Mutations in various phage genes can result in failure by otherwise temperate phages to produce lysogenic cycles. Mutations in the phage λ *cI* gene can result in complete loss of an ability to lysogenize. Mutations in other λ genes, particularly *cII* and *cIII*, too can result in substantial loss of that ability. Plaques are localized clearings of turbid bacterial cultures growing in or on agar-based media and these λ phage mutants form

clear plaques when plated on lawns of either non-lysogens or non-homoimmune lysogens. Plaques formed by wild-type temperate phages, by contrast, are turbid due to the establishment of phage-resistant lysogens over the course of plaque formation.

Phage λ *cI*, *cII*, and *cIII* mutants are still subject to superinfection immunity. Only certain double mutants in the λ operator sequences, O_L and O_R , which normally are bound by CI protein toward inhibition of phage transcription, are able to bypass superinfection immunity. As a result, these double mutants not only form clear plaques but do so even on lawns consisting of otherwise homoimmune lysogens.

The fact that it requires a double mutation for a homoimmune phage to bypass superinfection immunity is the strongest evidence that superinfection immunity provides prophages with a selective utility. Such evidence is only circumstantial, however, and it is entirely possible that the only true benefit of superinfection immunity is protection from that subset of phages that are neither clonally related nor heteroimmune. It is an open question, in particular, whether blocking productive infections by clonally related, superinfecting phages would provide an adaptive utility to immunity from superinfection.

Analogy, Practicality, and Conclusions

Phages are known to encode other mechanisms of resistance to phages besides superinfection immunity. Among these mechanisms is the somewhat analogous superinfection exclusion. Superinfection exclusion displayed by the non-temperate phage T4, as associated with the expression of two genes, 'immunity' and 'spackle', is a means by which superinfection itself is prevented following secondary phage adsorption. That is, superinfection exclusion is a postattachment block on phage-genome translocation into the bacterial cytoplasm, whereas superinfection immunity's

impact occurs after DNA translocation. Similar to superinfection immunity, it is uncertain whether superinfection exclusion's primary role is one of protection from subsequent phage infection since not only is that protection limited in breadth but also the gene products involved play important roles, at least with phage T4, in other aspects of phage infection.

Our general conclusion is that both superinfection immunity and superinfection exclusion may indeed play ecological roles in protecting phage infections from the disruption of superinfection, but evidence that these roles provide a selective benefit to the expressing prophages has not been robustly developed. Temperate phages, by displaying superinfection immunity, nevertheless, will tend to generate phage-resistant bacteria during their normal course of infection. That unquestionably is a problem given efforts to eliminate bacteria from environments using phages, that is, in the guise of what is known as phage therapy. For this as well as other reasons stemming from lysogen formation, the use of temperate phages is not recommended for phage therapy purposes.

Secondary adsorption is to an already phage-infected bacterium, such as of an already existing prophage. Superinfection exclusion blocks the initiation of superinfection by preventing phage entrance into the cytoplasm. Infection by homoimmune phages is blocked by superinfection immunity following phage-genome entrance into the cytoplasm. Heteroimmune superinfecting phages can successfully proceed with their infections, either productively or lysogenically, the latter resulting in polylysogens. The CI repressor, as indicated, is responsible for maintenance of the prophage state and generates superinfection immunity against homoimmune superinfecting phages. Induction occurs given the loss of the transcriptional repression necessary to sustain lysogeny, such as that mediated by CI. The result, given favorable conditions, is a productive infection, that is, maturation and then release of virion progeny (Figure 1).

Figure 1 Overview of secondary adsorption, superinfection, superinfection immunity, and so on.

See also: Analogy; Bacteria; Clever Mutant Isolation: Defining the Components of Cell Biological Processes; Fitness; Host-Range Mutant; Immunity: Phage, Plasmids, Transposons; Induction of Prophage; Lysis; Lysogeny; Mutation; Natural Selection; Operon; Phage (Bacteriophage); Phage λ Integration/Excision; Plaques; Promoters; Prophage; Repressor; T Phages; Temperate Phage; Transcription; Wild Type.

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