Bacteriophage P1 and Generalized Transduction in *E. coli*

**HISTORY**

Bacteriophage P1 was isolated in 1951 by Luigi Bertani. Transduction was discovered by Zinder and Lederberg during a search for genetic recombination in *Salmonella*. Expecting to find conjugative transfer similar to that they had previously observed in *E. coli*, they grew two mutant *Salmonella* strains together and selected for recombinants. Recombinants were indeed produced, but to the authors’ surprise, cell-to-cell contact was not required (as it is for plasmid-mediated conjugation). Rather, recombination proved to be mediated by a DNase-resistant filterable agent which was later demonstrated to be the bacteriophage P22. In 1955, Lennox reported that bacteriophage P1 could carry out generalized transduction in *E. coli*.

The generalized transduction system of P1 has been used extensively by *E. coli* geneticists for decades for the construction of strains and for the mapping of *E. coli* genes.

**VIRION**

The P1 virion exhibits classical bacteriophage morphology; it has an icosahedral head containing the phage DNA and a 220 nm long tail complete with tube, contractile sheath, baseplate, and six kinked tail fibers.

The P1 capsid is composed of 15 head proteins, 9 tail proteins, and 4 proteins of undetermined location. Oddly, populations of P1 produced by the same host cells contain virions with heads of different diameters. 80% of virions have a head diameter of 85 nm, while the other 20% have a diameter of 65 nm. Two head proteins, DarA and DarB, are interesting in that they protect the DNA from type I restriction systems after the DNA is injected into an infected cell.

**GENOME**

The DNA genome of P1 is about 94 kb and has been sequenced. There are about 120 genes that occupy 92% of the genome and are organized in 45 operons.

In the virion, the genome exists as a linear, double-stranded molecule. The genomes in any population of phages exhibit *circular permutation*. There is a relatively large (15 kb) *terminal redundancy*.

An important consequence of the extensive terminal redundancy of P1 DNA is that quite large pieces of foreign DNA can be inserted, provided that the insertions are in inessential regions. Viable P1 phage carrying transposon-mediated insertions of antibiotic-resistance genes have been isolated, and several of these have proven useful in laboratory manipulations of the phage. Our P1 strain (P1*clr100Cm*) carries a gene for resistance to tetracycline.
**ADSORPTION AND HOST RANGE**

The P1 receptor on the host cell is a terminal glucose of the lipopolysaccharide (LPS) core of the bacterial outer membrane. Addition of glucose residues to the LPS core requires a functional UDP-glucose pyrophosphorylase; galU mutants, which lack this enzyme, are P1-resistant. The LPS core polysaccharide is very widely distributed among many bacterial species. Therefore, a P1 virion can inject its DNA into a wide range of species. Although the P1 genome cannot replicate in these other species, P1 is potentially capable of moving DNA across species boundaries.

**INFECTION**

After infection, the DNA must circularize by homologous recombination between the terminal redundancies. This can be accomplished by either the host recombination system (RecA) or by a P1-encoded recombination system. (Note that this is strikingly different than the well-known mechanism for circularization of the Bacteriophage Lambda genome by ligation of single-strand "sticky ends".)

![Comparison of the Molecular Mechanism of Genome Circularization in Bacteriophage P1 and Lambda](image)

**LYSOGENY**

P1 is a temperate phage (i.e. it can form "lysogens" of its host) but is unusual in that the P1 genome in lysogens exists as an independent circular DNA plasmid-like element rather than incorporating into the host genome like the better-known phage Lambda. This means that P1 does not need a special site-specific recombination mechanism like the int/xis sytem in lambda.
But it does need to carefully coordinate replication and partition of its genome with the genome of the host cell.

P1 "plasmids" and lytically replicating P1 genomes replicate from different origins of replication, each subject to repression by specific repressor protein. Maintenance of lysogeny requires that replication from the lytic origin be completely repressed. P1 lysogens are extremely stable and are not easily induced. Preparation of transducing lysates from P1 lysogens has thus presented difficulties. These can be overcome by using a derivative of P1 with a temperature-sensitive mutation in the gene for the lytic repressor. Our P1 strain (P1clr100Cm) is an example of this.

PACKAGING BACTERIOPHAGE DNA

In the mid-1960s, Streisinger and his collaborators formulated an elegant model, dubbed "Headful Packaging" to account for the genesis of the circularly permuted, terminally redundant DNA molecules characteristic of bacteriophage T4 genomes. They proposed that the DNA substrate for packaging into the bacteriophage head is a multi-length DNA "concatemer", a polymer composed of multiple bacteriophage genomes arranged in tandem. If the length of the DNA packaged is determined by the capacity of the phage head (headfull packaging), and if the genome size is smaller than a headfull, terminally redundant DNA will be packaged. The necessary concatemeric substrates could arise in a variety of ways, such as through rolling-circle replication, recombinational multimerization, or end-to-end joining. There is substantial circumstantial evidence that the Streisinger model applies to the packaging of P1 DNA.

GENERALIZED TRANSDUCTION

Transduction is the heritable transfer of bacterial DNA from one cell (the donor) to another (the recipient) by a bacteriophage. Transducing bacteriophage particles are formed in donor bacterial cells during phage development. Generalized transducing phage particles carry a random fragment of host chromosomal DNA approximately the same length as the P1 DNA.

Generalized transduction should not be confused with specialized transduction. Specialized transducing phages, such as the well-known Bacteriophage Lambda, can transfer only certain bacterial genes located near the prophage insertion site in the bacterial genome. These bacterial genes may be incorporated into a single hybrid DNA molecule along with the phage genome. Such hybrid molecules are formed by rare aberrant excision of an integrated prophage from a lysogen. This means that specialized transduction can be mediated only by temperate phage whose lysogens involve reversible integration of the phage genome with that of the host cell.

Generalized transducing phage particles completely lack DNA originating from the phage genome and contain instead only bacterial DNA sequences. They arise when P1 genome-sized fragments of donor DNA are packaged into phage heads in place of phage DNA. The process is called generalized transduction because any part of the bacterial genome can be packaged and transferred in this way. Host cells that are infected by transducing phage particles receive only non-viral sequences, so they are not killed.

New genotypes in the recipient cells result from RecA-dependent (homologous) recombination, which can lead to the replacement of a recipient gene by an allele acquired from the donor genome via the transducing phage.
EVENTS IN THE DONOR CELL

Transducing phage particles are formed when the phage packaging mechanism seizes upon host DNA, instead of concatemeric phage DNA, as a packaging substrate. There is little or no specificity, so any portion of the host genome can be packaged with approximately equal frequency.

About 30% of the phage particles in a lysate contain host DNA rather than phage DNA. Given the relative sizes of the *E. coli* and P1 genomes, approximately 1 in 1,500 phage particles in a lysate will carry a given gene from the donor.

Oddly, the host DNA in transducing virions has a single molecule of a specific phage protein attached to each end. This protein is not attached to phage DNA in regular virions.

EVENTS IN THE RECIPIENT CELL

Once packaged within phage particles, transducing DNA is delivered to recipient cells as if it were phage DNA—linear DNA molecules are injected through the wall and cytoplasmic membranes into the cell. Three possible fates await them there: degradation by nucleases, recombination with homologous recipient DNA (formation of a stable transductant), and persistence within the cytoplasm in a form refractory both to recombination and degradation (abortive transduction). Only about 2% of the transduced DNA is recombined into the recipient genome.
No more than 10% of transduced DNA is degraded to its component nucleotides in recipient cells. This is in marked contrast to transformation, where most of the donor DNA is degraded by nucleases in wild-type recipients, and efficient transformation requires use of recipients that carry mutations that inactivate nucleases. (Like the endA1 mutation carried by DH5 stains of E. coli.)

Transduced DNA is also protected from degradation by restriction enzymes in the same way as phage DNA (see above).

Up to 90% of the transduced DNA remains trapped in the cytoplasm of the recipient cell as a stable form that does not replicate or recombine. Cells that contain such fragments of donor DNA are referred to as abortive transductants. Abortive transduction occasionally leads to the observation of barely visible colonies, each containing about $10^5$ cells. Each such colony contains one and only one cell which, upon restreaking on selective medium, is capable of forming a similar colony. This behavior can be understood if a stable, transcriptionally active but non-replicating DNA fragment is maintained in one cell at each cell division.

The ends of abortively transduced DNA, which should be prime targets for nuclease action, are apparently protected by the retention of the proteins attached at their ends. Furthermore, the retained protein apparently contributes to the formation of supercoiled circular fragments.

RECOMBINATION

Most homologous recombination in wild-type cells of E. coli is thought to proceed via the RecBCD pathway. RecBCD-mediated recombination is initiated when the RecBCD enzyme invades flush-ended double-stranded DNA fragments and separates the strands, with concomitant partial degradation of the 3' strand. Mediated by RecA, the product of RecBCD action, now with a single-stranded tail can "invade" the double helix of a homologous sequence to form recombinational intermediates.

This succession of events can lead to a double-stranded section of donor DNA replacing an equivalent length of recipient DNA. The length of the integrated fragment may vary up to the length of the donor DNA fragment (about 100 kb).
Bacteriophages are mixed with the B' cells and inject their DNA.

B' bacteria grow on this medium. B' bacteria do not grow on this medium.

When new viruses are released about 1 in 30 contain bacterial DNA. 1 in 50 of these contain any given gene, e.g. B'.

These B' cells can now grow on the selective medium.

In about 2% of these cells host B' DNA is replaced by transduced DNA, they are now B'.

About 1 in 1500 infected B' bacteria receive the B' gene.

The bacteriophage lysate is then added to the B' culture.
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