Generalized Transduction

MILLICENT MASTERS

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INTRODUCTION

Genetic transduction is the transfer, from one cell to another, of nonviral genetic material within a viral coat. Transduction was discovered by Zinder and Lederberg in 1952 (217, 218) during a search for genetic recombination in salmonellae. Expecting to find conjugative transfer, they grew two mutant strains together. Recombinants were indeed produced, but to the authors' surprise, cell-to-cell contact was not required. Rather, recombination proved to be mediated by a DNase-resistant filterable agent which was later demonstrated to be identical to the bacteriophage P22. In 1955, Lennox (103) reported that, similarly, the temperate coliphage P1 could also act as a vector to carry out what has come to be termed generalized transduction.

Transducing bacteriophage particles are formed in donor bacterial cells during phage development. They are of one of two types, distinguished by the nature of the DNA molecule which they carry. Generalized transducing particles, competent to mediate generalized transduction, carry a fragment of host DNA; specialized transducing particles, able to carry out specialized transduction, contain both host and viral DNA sequences as part of a single molecule. Such hybrid molecules are formed, in vivo, by aberrant excision of a prophage (or other recombinational event) to yield DNA molecules which can be both replicated and packaged. Specialized transducing particles arise at low frequencies, although, once identified, they can be propagated, with the aid of a helper phage if required, to yield high-frequency transducing lysates. Specialized transductants are likely to be lysogens which are diploid for the transduced host markers. The transduced DNA will have been added to the recipient genome and the transductants are able to produce transducing particles in their turn. The range of host markers which can be transduced in this fashion is limited to those near prophage insertion sites or other sites of recombination between phage and host sequences unless the transducing particles have been engineered in vitro to contain other DNA. Specialized transduction is treated in detail in chapter 131 of this volume.

The products of generalized transduction are quite different from those of specialized transduction. Generalized transducing lysates, although composed primarily of infectious phages, contain, in addition, a small proportion of transducing particles which, on subsequent infection of a recipient culture, deliver a fragment of host DNA to a minority of cells. Generalized transducing particles completely lack DNA originating from the viral vector, containing instead only sequences of host origin. They arise when viral-genome-sized fragments of donor DNA are packaged into phage heads in place of viral DNA. The process is called generalized transduction because any part of the host genome can be packaged and transferred in this way. Provided that transduced cells have not been coinfected with normal viral particles, they will have received only nonviral sequences. Stable transductants result from RecA-dependent replacement of recipient by transduced DNA, and progeny cells will thus be haploid for the transduced region and nonlysogenic. Generalized transduction need leave no evidence, other than a possible exchange of alleles, that it has occurred. Figure 1 provides an overview of generalized transduction.

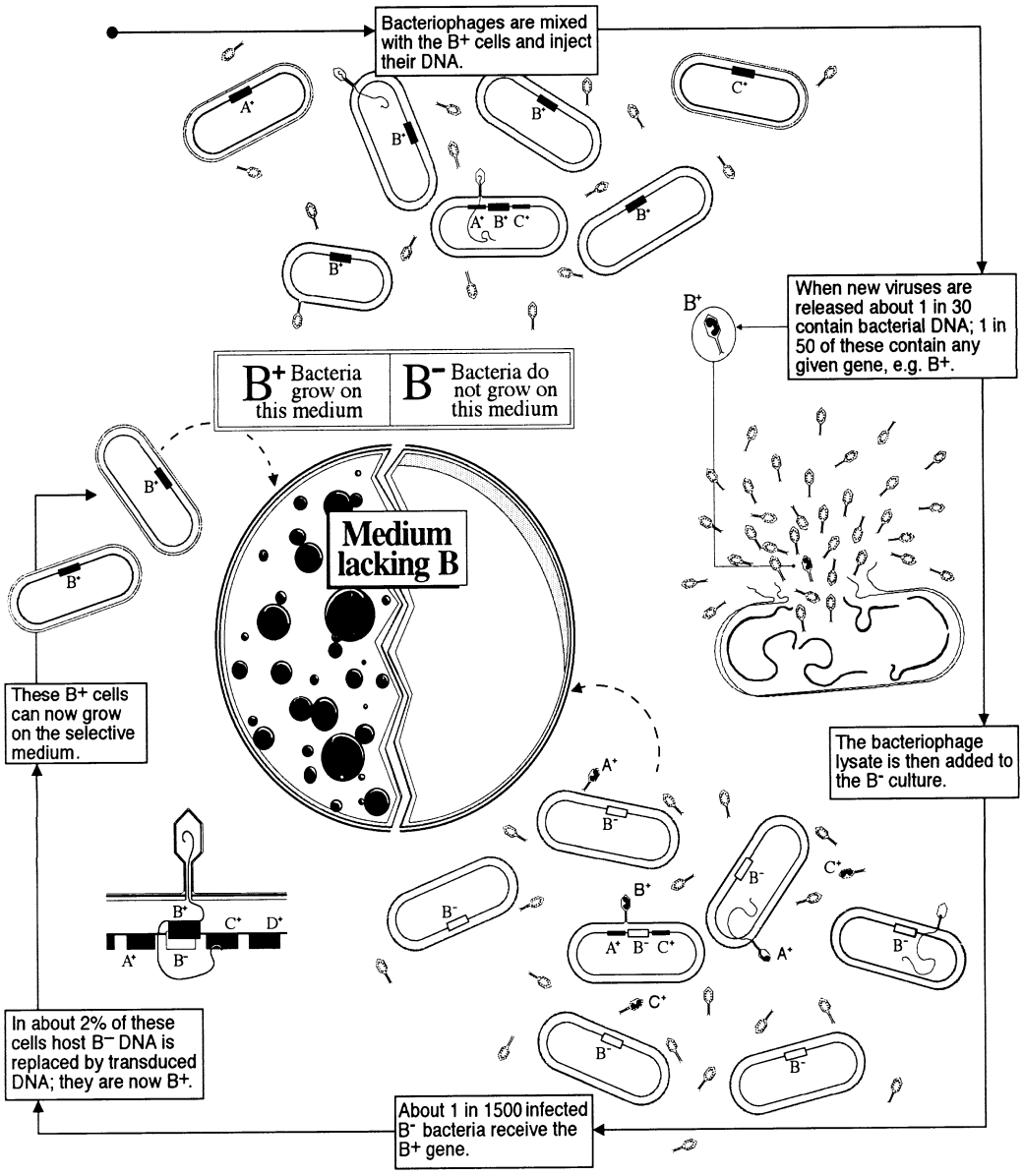

FIGURE 1 Overview of the transduction process. The picture, which proceeds clockwise from the upper left, applies to all natural transducing systems, but numerical information is based on the behavior of the P1-E. coli system. It shows the production of a P1 lysate on a prototrophic strain and its use to transduce B^+ to a B^- recipient. Donor and recipient are otherwise isogenic. This is indicated by the inclusion of A^+ and C^+ in representative cells.

The bacteriophages P22 (for *Salmonella typhimurium* [official designation, *Salmonella enterica* serovar Typhimurium]) and P1 (for *Escherichia coli*) remain the vectors of choice for generalized transduction. The P22 phage coat can hold about 44 kb, a bit less than 1% of the host's chromosomal DNA; P1 is larger, with a capacity of over 2% of the host's DNA. Because of the low frequency with which transducing particles occur in lysates (see below), a single recipient cell is unlikely to receive more than one transducing particle; cotransduction (the inheritance of more than one donor marker by a single recipient cell) is thus possible only if the markers selected are close enough to be carried within a single transduced fragment. Because of this, cotransduction has been a powerful method for gene mapping and still continues to be widely used for strain construction. Although generalized transduction appears to be an accidental concomitant of phage infection rather than an evolved biological process, this has not prevented it from taking its place alongside plasmid transformation (another unevolved process) as one of the two currently most used methods for gene transfer between *E. coli* or *S. typhimurium* strains.

Several comprehensive reviews of generalized transduction have appeared in the last decade, all as chapters in books. These include an article by Masters (118), an extremely detailed, 150-page, near-monograph by Yarmolinsky and Sternberg (212) which covers all aspects of phage P1 biology, a more recent article by Sternberg and Maurer (178) which includes laboratory protocols, and, of course, the excellent chapter by Paul Margolin in the first edition of this book (114). Two reviews on P22 biology also include discussions of transduction by this phage. The comprehensive 1978 review by Susskind and Botstein (184) remains extremely useful; a more recent review by Poteete (138) complements it. I shall draw heavily on all of these sources but will not attempt to duplicate here all the information which appeared in Margolin's chapter in the first edition of this book.

THE PREFERRED TRANSDUCTIONAL VECTORS: PHAGES P1 AND P22

Life Cycles and Related Phages

Bacteriophages P1 and P22 remain the preferred vehicles for generalized transduction in *E. coli* and *S. typhimurium*, respectively, and this chapter will focus particularly on these phages and the mechanisms by which they transduce DNA. For detailed coverage of the P1 life cycle, see reference 212.

P1 was initially isolated in 1951 from *E. coli* by Bertani (13), using *Shigella dysenteriae* as an indicator. Variants which reliably form plaques on the *E. coli* K-12 strains favored by geneticists were required to transform it into the laboratory workhorse that we use today. An early isolate, still commonly used, is P1*kc* (103) (so called because the plaques formed are clearer than those formed by its parent rather than because it is unable to lysogenize infected cells). P1 is related to the similar bacteriophages P15B (77) and P7 (169, 216). These temperate phages share the unusual property of forming plasmids on lysogenization but differ, of course, from other plasmids in the mode by which they are tranferred between cells. Rather than encoding or exploiting conjugative systems, P1, the plasmid, lyses its host and finds a new one in its guise as P1, the bacteriophage. Thus, P1, in contrast to integrating temperate phages, is not normally an integral part of the bacterial chromosome.

P1 plasmids (2) and lytically replicating P1 phages (see reference 212 for a discussion) replicate from different, but adjacent, origins, each subject to appropriate repression by specific repressor molecules. Maintenance of lysogeny requires that replication from the lytic origin be completely repressed, and as expected, cells lysogenic for the P1 plasmid are immune to superinfection by P1 phages. P1 lysogens are extremely stable. Although copy number is low, partition at division is accurate and few cells cured of plasmid can be detected (3). P1 lysogens are also not easily induced. Although there have been reports that induction with UV or radiomimetic agents is possible (93, 125), others have been unable to reproduce these results (110, 212). Preparation of transducing lysates from P1 lysogens has thus presented difficulties. These have been overcome by using a derivative of P1, P1*vir*^{s} (110), to infect P1*kc* lysogens. P1*vir*^{s} constitutively produces a phage antirepressor enabling it to grow on P1 lysogens and preventing it from

forming them (see reference 163 for a discussion). Alternatively, the heteroimmune phage P7 (31, 200), which can grow on P1 lysogens, can be used to make transducing lysates. Another approach is to use a temperature-sensitive specialized transducing phage such as P1Cm*c*1*ts* (147) to replace P1*kc* by selecting for chloramphenicol resistance and testing for temperature sensitivity. Transducing lysates can then be prepared by induction of P1Cm*c*1*ts* at 42°C.

The P1 genetic map, vegetative or prophage, is linear, although the phage and prophage replicate as circles and the map is presented as a circle (211). This is because P1 specifies a site-specific recombination system, the *lox-cre* system, which, by efficiently promoting recombination between pairs of *loxP* sites within the same molecule, effectively masks linkage between physically close markers separated by the recombination site (176).

P22, in contrast, is a member of the well-studied group of lambdoid phages (for a review, see reference 184). Its gene organization is similar to that of lambda, with genes of related function clustered on the genetic map. Because of this "cassette" organization and because there is considerable DNA-DNA homology between the two phages, P22-lambda hybrids can easily be made. P22 has a circular vegetative and a linear prophage map; the linear prophage map has its origin in the fact that P22, like lambda, lysogenizes via site-specific integration into the continuity of the host chromosome at a site, *ataA*, close to *proA*. This site, also present and usable in *E. coli* (where is is called *attP22*), is most probably within the *thrW* gene which encodes a threonine tRNA (106). P22 lysogens can be induced, in $RecA^+$ cells, by UV or radiomimetic agents, with the consequent RecA-mediated cleavage of the *c*1 repressor leading to lytic phage development.

Adsorption and Host Range

The P1 receptor on the host cell is a terminal glucose of the lipopolysaccharide (LPS) core of the bacterial outer membrance. Addition of glucose residues to the LPS core requires a functional UDP-glucose pyrophosphorylase; *galU* mutants, which lack this enzyme, are P1 resistant (53). In *S. typhimurium*, the LPS core contains the oligosaccharide-mediated O-antigenic specificity necessary for P22 adsorption. P1 will not adsorb to wild-type *S. typhimurium* but will adsorb to rough derivatives that have lost parts of the oligosaccharide side chain (131). Such mutants can be used in interspecific crosses or as recipients in P1-mediated transduction between *Salmonella* strains. P22 appears to adsorb to only a limited range of *Salmonella* spp. Although P22 can replicate within and lyse *E. coli* K-12 if introduced on a plasmid, it cannot give rise to plaques on *E. coli* K-12 because *E. coli* does not encode the enzymes necessary for O-antigen synthesis. Introduction on a plasmid of the genes necessary to permit O-antigen synthesis can transform *E. coli* K-12 into a derivative permissive for P22 propagation (126). Yarmolinsky and Sternberg (212) present a table showing the extensive range of bacteria into which P1 can inject its DNA. As well as a wide range of enterobacterial species, such distantly related species as *Myxococcus xanthus* and several *Pseudomonas* species are included. Although productive infections are seldom obtained from nonenterobacterial species, DNA originating from permissive hosts, such as transposons intended as mutagenic agents, can be successfully delivered to these species by P1 infection.

Morphology and DNA

P1 exhibits classical bacteriophage morphology; it has an icosahedral head and longer tail (ca. 216 nm in length) complete with tube, contractile sheath, baseplate, and six kinked tail fibers (195, 198). Lysates contain particles of more than one size; these have tails of the same length but heads of different diameters. The two main classes are P1B, with a head diameter of about 85 nm, which normally comprises about 83% of the population, and P1S, with a head diameter of 65 nm, which comprises most of the remainder (80, 195, 197). P1*vad* mutants, deficient in internal head proteins, can produce equal numbers of P1B and P1S (73, 76).

Plaque-forming P1B particles contain double-stranded, linear, terminally redundant, circularly permuted DNA. A headful of P1B DNA (i.e., the amount of DNA which can be encapsidated within a particle of a particular size) is about 66 MDa in mass and comprises 100 kb of DNA with a GC/AT ratio of 0.46. P1 DNA is thus less dense than that of its host (78). (*E. coli* DNA is about 51% GC.) Ikeda and Tomizawa estimated the extent of terminal redundancy at 12% (81); Yun and Vapnek in 1977 (216) reestimated it to be about 8%. Genome size, calculated from the length of prophage DNA, is about 90 kb (80, 216). Terminal redundancy was demonstrated (81) by denaturing P1 DNA and allowing it to reanneal. The products of this reaction were circles with the terminally redundant regions visible as unannealed "bushes." Measurement of the lengths of these bushes permitted the calculation of the degree of terminal redundancy. The distance between the bushes in individual reannealed molecules depends on the degree of permutation between the annealed single strands. This distance varied over a wide range between molecules, indicating that circular permutation can be extensive, perhaps as high as 40 to 50%. P1S particles contain 40% as much DNA as do the larger P1B DNA molecules; P1S DNA, although circularly permuted (196), is not terminally redundant. Since P1S particles carry only about 45% of the P1 genome, they cannot productively infect cells at low multiplicities. At high multiplicities, however, productive infection can occur, confirming that circular permutation among the DNA molecules of these particles must be great (80). A consequence of the extensive terminal redundancy of P1 DNA is that quite large pieces of foreign DNA can be tolerated, provided that the insertions are in inessential regions. Plaque-forming P1 particles carrying multiple drug resistances (73), resulting from insertions into an IS*1* site native to P1 (74), have been isolated, and certain of these have been extremely useful for the preparation of generalized transducing lysates (see above).

The P22 virion is much smaller than is that of P1. It consists of an icosahedral head, 60 nm in diameter (47, 208), with a very short 16-nm tail from the center of which a 20-nm spike or fiber protrudes (18, 37, 82, 208). The virion contains nine different protein species: a head protein (22), a tail protein (18), four less abundant proteins at the point of tail attachment (67), and three minor (70, 83, 140), possibly internal, proteins which appear to be essential for successful DNA injection. One of these, gp16, has recently been implicated in the establishment of P22 abortive transductants (N. Benson, personal communication). A headful of P22 DNA, about 43.5 kb in length, is packaged regardless of genome size (the P22 genome size has been altered by insertion and deletion) (85, 192, 193). The native P22 genome has most recently been measured to be 41.8 kb long (24); a P22 headful of DNA is therefore normally 4% terminally redundant. Circular permutation of P22 is less than that of P1 DNA and is limited to about 20% of the genome (193). (This will be discussed further in the following section.)

Productive infection with either P1 or P22 requires homologous recombination within the infected cell to convert the injected terminally redundant linear phage DNA into a circular replicating form. This can be accomplished by the host recombination system in $RecA^+$ recipients or by a phage-encoded recombination system; thus, the P22 *erf* gene product is essential for phage development in *recA* hosts (17, 201, 207). The fact that it is not needed for phage growth after induction of a lysogen, a situation in which the *int-xis* system yields a circular product, suggests that its role is to circularize the terminally redundant infecting DNA (17). P1 molecules which contain two copies of *loxP* can be circularized by the *cre* gene product, permitting establishment of lysogeny, and presumably also vegetative growth, to proceed in the absence of host-encoded RecA (176).

PACKAGING BACTERIOPHAGE DNA

Although the reviews cited above also discuss packaging, the packaging of P1 (23) and P22 (172) DNAs has been specifically reviewed in a volume published in 1989. These reviews and another (14), which discusses double-stranded DNA phage packaging more generally, will be drawn upon here.

Headful Packaging

In the mid-1960s, Streisinger and his collaborators formulated an elegant model to account for the genesis of the circularly permuted, terminally redundant DNA molecules characteristic of bacteriophage T4 (182, 183). They proposed that the DNA substrate for encapsidation into the bacteriophage prohead is a DNA concatemer, a polymer composed of bacteriophage genomes arranged head to tail. If the length of the DNA packaged is determined by the capacity of the phage head (headful packaging) and if the genome size is smaller than a headful, terminally redundant DNA will be encapsidated (Fig. 2). The necessary concatemeric substrates could arise in a variety of ways, such as through rolling-circle replication to yield sigma forms, recombinational multimerization of theta forms, or end-to-end joining of the incomplete replication products of terminally redundant linear molecules, produced after the removal of the 5′-terminus replication primers. The Streisinger model could, of course, be extended to the packaging of P1 and P22 DNAs, and earlier indirect supporting evidence has been confirmed by more recent molecular studies.

A prediction of the headful packaging model is that the extent of terminal redundancy will vary with genome size. For P22, this was tested physically by using electron microscopy to examine homoduplexes of phage DNA which had been denatured and reannealed (192). Two deletion derivatives, of 5 and 14%, respectively, were found to have the anticipated increased terminal redundancy, while an insertion mutant with an "oversized" genome lacked terminal redundancy (and the ability to initiate productive infections in singly infected cells [SIC]). P1 phages with oversized genomes, unable to form plaques on single infection, regain plaque-forming ability by deletion (75).

The Streisinger model also explains circular permutation. If headfuls are cut at random from concatemers, the distribution of ends vis-à-vis the genetic map will also be random and circular permutation will be complete; T4 DNA appears to be packaged in this way (183). If, however, headful cutting starts at a particular sequence and subsequent cuts are sequential, the degree of circular permutation will depend on the degree of terminal redundancy and the number of headfuls packaged from each concatemer. Using electron microscopy techniques, Tye et al. demonstrated (193) that for P22, all of the ends are clustered within only 20% of the genome. This is as would be expected for a phage with limited terminal redundancy, packaged sequentially but with limited processivity, from a fixed start site sequence on the concatemer. Consistent with this, a P22 deletion derivative with 7% terminal redundancy (instead of 3 to 4%) had ends distributed over 60% of the map. Interestingly, a derivative with a large deletion, resulting in 16% terminal repetition, tended to have ends clustered in discrete regions of the map, well displaced from one another. These could represent the ends of the first, second, etc., sequential headfuls, made distinguishable in this case by the large displacement between ends caused by the length of the terminal repetition (Fig. 2).

These electron microscopy observations were supported by restriction endonuclease analysis of packaged DNA. Jackson et al. (85, 87) analyzed both wild-type DNA and that of derivatives with genome sizes altered as a result of DNA insertion or deletion. They observed that a particular band, common to each phage digest, always occurred in submolar quantity. There was only a single such band; its position on the circular genetic map, in a region of probable enhanced recombination, was consistent with its being at the end of the linear chromosome. Additionally, it was predicted that for an insertion mutant with a genome length almost identical to the length of packaged P22 DNA, all headful terminations would occur in a particular restriction fragment, leading to its loss from the digest. This indeed proved to be the case, allowing these authors to conclude that packaging begins at a specific site, which they termed *pac*, which is located within the missing fragment. The submolar fragment found in digests of unmutated phage DNA must thus contain one *pac* end and one restriction site end. It was present at about one-third the molarity of the other fragments, suggesting that processive packaging from concatemers continues for only about three consecutive headfuls. When wild-type P22 DNA was digested with *Eco*RI, no additional submolar fragments were observed, suggesting that the terminus of even the first headful and certainly the starts of subsequent headfuls may not be very well defined

(thus leading to difficulty in visualizing the very small bands expected). To circumvent this problem, an insertion mutant lacking terminal redundancy and predicted to yield long terminal bands, easier to resolve on gels, was studied. Submolar bands originating from the ends of second and third headfuls were duly found, confirming the hypothesis that sequential packaging of P22 proceeds for about three headfuls.

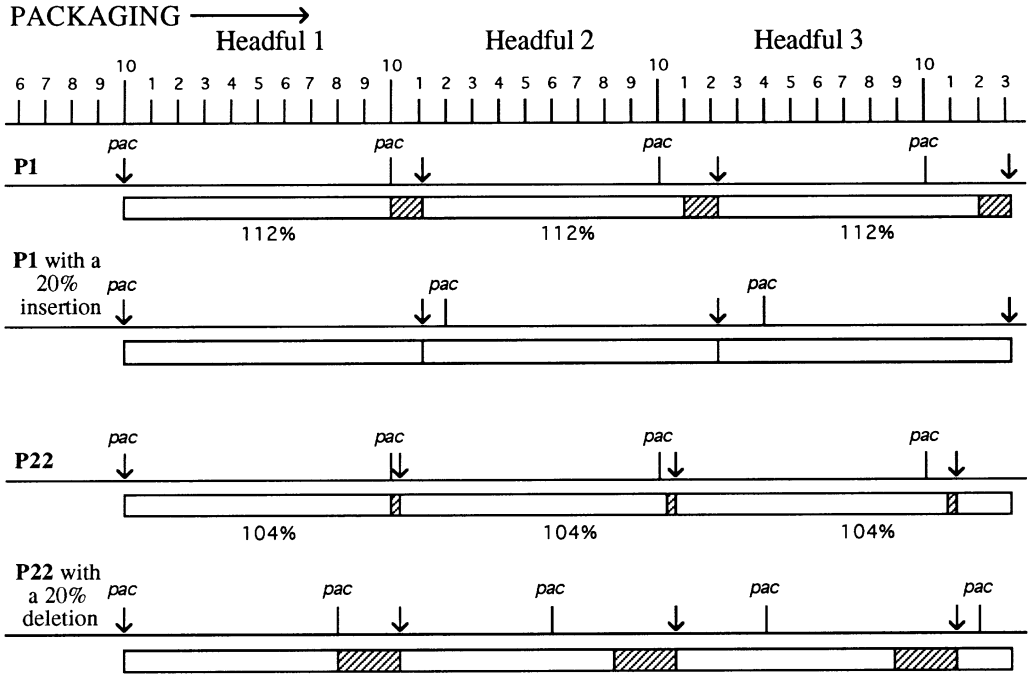

FIGURE 2 Headful packaging and terminal redundancy of phage DNA. Terminal redundancy for P1 is large, and the ends of packaged DNA are distributed over much of the genome (assuming that an average of four headfuls are packaged per concatemer). A 12% insertion would abolish terminal redundancy and result in unique ended DNA. For insertions greater than 12%, such as the 20% illustrated here, circular permutation will again be apparent. P22 terminal redundancy is small, and the DNA ends are therefore clustered together on the genome. A large deletion results in the introduction of a corresponding large terminal redundancy and ends which are distributed over the genome. The uppermost line in the diagram is a scale in which 10 is the length of the wild-type phage genome. For each of the four examples, the upper line represents the concatemeric packaging substrate, with the distance between *pac* sites used as a marker to indicate genome length. The initial packaging cut occurs near the leftmost *pac* site; arrows indicate this and the subsequent cust sites which define individual packaged molecules. Terminally redundant DNA is represented by hatched bars.

Bächi and Arber (4) performed a similar restriction site analysis for P1 phage DNA. They too found a fragment present at about one-third the molarity of other fragments, suggesting that for P1, sequential packaging also proceeds for about three headfuls. An analysis of mutant DNAs corresponding to that done for P22 was not performed, and submolar fragments corresponding to the ends of the second and higher-order headfuls were not identified. Comparison of the restriction map obtained with that of the circular prophage DNA allowed Bächi and Arber to locate the position of the site from which P1 packaging was presumed to begin; this is at 92 units on the genetic map, adjacent to capsid subunit and maturation genes.

Molecular Mechanisms Involved in Packaging

P22. Encapsidation of P22 DNA (reviewed in reference 23) requires only five phage-encoded proteins. Three of these are structural components of the prohead; the remaining two, the products of the contiguous genes *2* and *3*, are transiently associated with the prohead but not present in the final virion. *pac* is located within gene *3* (5, 25, 26, 202); gp2 and gp3, but not the procapsids, are required for the generation of the specific ends found in mature DNA (100). Closer analysis has shown that the sequences at these sites of cutting (called *end* sites) are located within a 120-bp region which also contains *pac* (95, 202), which has now been redefined as the recognition (as opposed to the cutting) site for packaging initiation (26). Six *end* sites, separated by 20 or 40 bp, have been identified (5, 25). Recent work (S. Casjens, personal communication) has identified *pac* as a sequence, 17 bp in length, located in the middle of the *end* region. It seems likely that gp2 and gp3 both recognize *pac* and are responsible for cutting at *end;* in their absence, both proheads and DNA concatemers accumulate (18).

DNA, possibly still associated with gp2 and/or gp3, is thought to enter the procapsid through a hole formed by the portal protein. The mechanism by which this occurs has not yet been defined. During the course of entry, the scaffolding protein (an internal protein thought to determine prohead shape) is expelled from the structure, and with an increase in diameter, the prohead is transformed into the mature head. After sufficient DNA has entered the head, a nuclease, still undefined, carries out the terminating cut. The identity of the gauge that measures DNA by the headful is also unknown, although recent work implicates the portal protein itself in this process (28). Three further virion proteins are necessary to stabilize the particle and lock the DNA within it (181). With their addition, the disassociation of gp2 and gp3, and the attachment of the tail protein, phage particle assembly is complete.

P1. The P1 capsid is more complex than is that of P22. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of P1 particles detected 15 head proteins, 9 tail proteins, and 4 proteins of undetermined location (197, 198). The head proteins include four dispensable internal proteins. Two of these, DarA and DarB (76), are interesting in that they appear to protect the contents of the phage head from type I restriction systems in the recipient, while another internal protein, Vad (for viral architectural determinant), is required for the synthesis of normal amounts of P1B (73, 76). In its absence, equal numbers of P1B and P1S particles are produced.

The cutting of P1 DNA concatemers for packaging is dependent on the products of two cotranscribed and cotranslated genes, *pacA* and *pacB* (166). P1 amber mutants with defects in these genes cannot cleave concatemers. In contrast, phage mutants which are unable to produce late gene products are still able to cleave in the *pac* region, suggesting that association with proheads is not required for their activity (173).

Sternberg and Coulby mapped *pac* to the 620-bp *Eco*RI restriction fragment 20 (174), confirming the location deduced by Bächi and Arber (4). They then investigated in vivo cleavage at *pac* in an elegant series of experiments (173). By cloning *pac* into lambda, to make a lambda-P1 hybrid which could be integrated by lysogenization at *att*-lambda, it was possible to insert *pac* into the continuity of the bacterial chromosome. *pac* was also cloned into a plasmid vector and transformed into P1-sensitive cells. After infection of these strains with P1, DNA was extracted from cells and Southern hybridization was used to monitor the fate of *pac*-containing DNA independently of actual packaging. It was shown that after cutting at *pac*, the end of the cut destined to be incorporated into particles, particularly when originating from the chromosome, appeared to be protected from nuclease digestion but that degradation at the other end quickly occurred. (Inactivation of the host RecBCD improved the recovery of plasmid-encoded *pac* ends.)

Transduction experiments, using the chromosomal integrants as donors, extended these observations. Chromosomal markers to one side of the inserted prophage, but not the other, showed greatly enhanced transduction frequencies (to be discussed further below), leading to the conclusion that the protected DNA end acts as the origin of sequential, unidirectional packaging. Since enhanced transduction frequency was observed for markers more than 10 min (five headfuls) from the insertion site, it appeared that more DNA

could be packaged from the chromosomal substrate than is normally packaged from P1 concatemers. This, in turn, suggests that processivity may be limited, at least in part, by the length of the substrate DNA.

As is the case with P22, P1 packaging initiates within one of the P1 *pac* genes (174). A functional cleavage site is contained within a 162-bp segment of the small P1 *Eco*RI fragment 20. Cleavage occurs centrally within this sequence, most often at one of two sites separated by about a half turn of the double helix. *pac* (the cleavage recognition region) contains seven repeats of the sequence TGATCA, four to the left and three to the right of the cut site (175). Effective cutting requires the integrity of six of the seven repeats. The repeats contain the sequence GATC, the methylation site of the *E. coli dam* methylase; effective *pac* cutting, both in vivo and in vitro, requires that the GATC sites be methylated. Methylation can be carried out either by a host or by a P1-encoded methylase; growth of a P1 Dam^- phage is normal in a dam^+ but much reduced in a *dam* host. Yarmolinsky and Sternberg suggest a role for *dam* methylation in P1 packaging (212). They point out that were cutting to occur at all *pac* sites, genome-length fragments would result and the terminal redundancy necessary for cyclization in the recipient cell would be precluded. If methylation were to be limited by an inhibitor which is able to bind to and protect the hemimethylated DNA produced at replication, further methylation of protected sites would be prevented and later cutting would be limited to the minority of sites which escape protection and become fully methylated. Consistent with this hypothesis is the demonstration that uncut potential cleavage sites in the DNA of mature phages are undermethylated and cleavage resistant (175). In vitro methylation converts them into substrates cleavable by PAC enzymes. Successful P22 packaging obviously also requires that not all *pac* sites be utilized. Since a high frequency of GATC sequences is not characteristic of the P22 *pac* region, this cannot be the mechanism used to limit *pac* site utilization.

THE FORMATION OF TRANSDUCING PARTICLES: EVENTS IN THE DONOR CELL

DNA in Transducing Particles

Transducing particles are formed when the phage packaging mechanism seizes upon host DNA, instead of concatemeric phage DNA, as a packaging substrate. The elegant experimental work which first permitted this conclusion was completed by Ikeda and Tomizawa in 1965 (78, 79). They studied the formation of P1 transducing phages by density labeling the DNA of donor cells with 5-bromouracil (BU) before infection and replacing the BU with thymine at the time of infection. Lysates were centrifuged through CsCl density gradients, and infective and transducing particles were assayed in each fraction (78). In control experiments in which either thymine or BU labeling was used throughout, transducing and infective particles had similar but distinguishable densities which reflected the differing GC/AT ratios of the two types of DNA. When, however, the medium was altered at the time of infection, density measurements clearly showed that transducing particles contained only DNA which had been present before infection; conversely, the infective particles contained only DNA made postinfection.

These experiments used P1*vir*. Infection with this phage results in the immediate cessation of host chromosome replication, facilitating the interpretation of labeling data obtained after a concomitant medium shift. Host DNA replication continues after infection with the commonly used P1*kc*, making it less suitable for this type of experiment. Differential labeling of infective and transducing particles with radioactive label during infection was used to estimate the proportion of transducing particles within P1*vir* lysates as 0.3%.

Density analysis of the DNA within phage particles revealed a surprising anomaly (79). Since phage P1 and *E. coli* have DNAs of different densities, it was anticipated that the DNAs isolated from infectious and transducing particles would be correspondingly different. In fact, both DNAs had the same density, with that of the transducing particles being lower than expected. This was shown to be the result of the association of transducing, but not infectious, DNA with a protease-sensitive component; it was further estimated that 500 kDa of protein is associated with each DNA molecule. The association of transducing

DNA and protein was confirmed and expanded upon by Sandri and Berger in 1980 (153). During an investigation into the fate of transducing DNA in the recipient cell, they found that a substantial fraction (>75%) of the transduced DNA appeared to adopt a circular conformation maintained by an attached protein. This protein has not been studied further. It has been suggested that the protein may be packaging enzyme which remains associated with the transducing DNA after completing cleavage (212); this, however, remains to be established.

P22 transducing phages, like those of P1, contain primarily host DNA synthesized before infection. The first evidence that this is so was reported by Starlinger in 1959 (170). He found that when P22 was grown on ^{32}P-labeled bacteria in a nonradioactive medium, transducing activity was subject to radioactive decay but infectivity was resistant. Later, both Schmieger (155, 156) and Ebel-Tsipis et al. (48) performed, with the P22-*Salmonella* system, experiments similar to those of Ikeda and Tomizawa, using, respectively, (i) BU and (ii) ^{2}H, ^{13}C, and ^{15}N to density label host DNA. Both groups concluded that at least 90% of the DNA in transducing particles is made before infection. Ebel-Tsipis et al. (48) suggested that the small density differences observed between host and transducing particle DNA might be explained by the continuation of host DNA replication after infection or a preference for encapsidation of chromosomal regions lighter than average. Schmieger (156) sought, but was unable to find, evidence for a protein analogous to that responsible for reducing the density of P1 transducing DNA. He proposed a recombinational model in which covalently linked host and phage DNAs were substrates for encapsidation as an explanation for the observed density of P22 transducing DNA. The lack of dependence of transducing particle formation on either the host *rec* or the phage *erf* general recombination systems makes this interpretation unattractive (48, 184).

Ebel-Tsipis et al. (48), confirming similar earlier observations by Sheppard (164) and Roth and Hartman (148), among others, noted that transducing phages differed in density, depending on the host markers carried. This is in contrast to P1 transducing phages and requires both that there be significant position-dependent heterogeneity in DNA density on the *Salmonella* chromosome and that the phages that transduce particular markers be relatively uniform in DNA sequence (i.e., that they start and end with the same sequences). It was noted that *gal* transducing phages formed two populations distinct in density as might be expected if there were two, but only two, unique overlapping *gal*-containing sequences that can be encapsidated (48). These observations are in accord with those of Ozeki (133), who had concluded that all phages transducing certain linked markers had a uniform genetic composition. His results, however, cannot be generalized; other linked markers were shown to be transduced by a heterogeneous collection of overlapping fragments (50, 136).

These density transfer experiments also allowed a physical estimate of the fraction of particles that contain transducing DNA; they were calculated to form about 2% of the total particles in a P22 lysate.

Encapsidation of Transducing DNA

How then are transducing particles, containing host DNA but free of bacteriophage DNA, formed? Two alternatives have been considered. The first is that chromosomal DNA becomes fragmented after infection, perhaps through nuclease action, into pieces of appropriate size, and that these pieces are then encapsidated. This has been shown not to be the case, at least for P22; phage genome-sized fragments of host DNA do not accumulate after infection, even in mutants with enhanced transducing ability (157, 161). The more plausible alternative is that large lengths of chromosomal DNA (perhaps entire chromosomes) act as substrates for encapsidation in place of phage DNA concatemers. How are they recognized as substrates? Again there are two possibilities. The chromosome may contain sequences which sufficiently resemble bacteriophage *pac* sites for the phage *pac* recognition system to accept them as substrates. Alternatively, packaging might occur in a non-sequence-specific manner either from DNA ends or as a consequence of nonspecific cutting by packaging enzymes. P1 and P22 differ here; accumulated evidence clearly favors the *pac* site mechanism for P22 transducing particle formation but a non-sequence-specific

mechanism for the formation of P1 transducing phages.

P1: Evidence for Lack of Specificity

The earliest evidence suggesting lack of specificity in packaging P1 transducing DNA was the observation by Ikeda and Tomizawa (78) that all transducing particles, irrespective of the markers assayed (five separate markers were tested), had identical density profiles. If DNA density differs among chromosome segments of the size encapsidated in a P1 particle (and it does; analysis of the 0.5 Mb of available continuous sequence from the 80- to 90-min region reveals that P1-sized fragments vary in GC content from 51 to 53% [M. Masters and J. F. Collins, unpublished results]), it can be inferred that the particles carrying a particular marker are not likely to contain DNA identical in sequence, but rather a set of overlapping segments which have the selected marker in common. Consistent with this is the fact that no closely linked pairs of markers have been identified with cotransduction frequencies very much lower than anticipated, as would be found if they were seldom within the same transducing fragment. Lack of packaging specificity can also be inferred from the observation that the frequencies with which individual markers can be transduced by P1 do not vary over a very wide range; in several studies (21, 199), a 10- to 20-fold range in frequencies, from $1 \times 10^{-6 \text{ to } 2 \times} 10^{-5}$ per infectious particle, was typically found. Masters (117), in a more extensive study, measured transduction frequencies for 26 markers and found that except for markers close to *oriC* (to be discussed further below), transduction frequencies varied over only a 10-fold range. Part of this variation originates in the gradient in gene dosage, from replication origin to terminus, characteristic of the exponentially growing donor cells used to prepare lysates, and much of the remainder is due to recombinational selectivity (see below).

Hanks et al. (60) used a quantitative hybridization technique to compare physically the frequencies with which 16 markers are encapsidated by P1*kc*. Packaging of most markers varied within a threefold range, although two chromosomal regions, the 2-min region and the region including *rpoBC*, exhibited threefold-higher encapsidation frequencies. This is reflected, for markers in the 2-min region, in correspondingly high transduction frequencies. These authors also observed, within transducing DNA, some DNA bands of sizes not found in the corresponding chromosomal restriction digests. These could indicate the presence of many (since they were uncovered with most probes), occasionally used (since they were of low intensity), specific start sites for host DNA encapsidation. The fraction of phage which were transducing was estimated from these data at 6%, considerably higher than the 0.3% estimated for P1*vir* (78; also see above).

The lack of obvious selectivity in packaging transducing DNA and the fact that it is so much less likely than phage DNA to be packaged make it improbable that packaging occurs from bona fide *pac*-like sites on the chromosome. This inference is supported by the inability to detect hybridization between chromosomal DNA and a *pac* DNA probe (60; N. Sternberg, cited in reference 212). When P1 *pac* is inserted into the chromosome (173; M. Hanks, Ph.D. thesis, Edinburgh University, Edinburgh, Scotland, 1986), transduction frequencies of markers to one side of the point of insertion can be elevated by as much as 80-fold; thus, we can conclude that any pseudo-*pac* sequences that might occur on the chromosome are no more than ca. 1% as effective as the phage sequence.

Harriman, in 1972 (61), performed an elegant single-burst analysis of transducing particle formation. By using a donor strain lysogenic for three bacteriophages, the lambdoid phages lambda, 21, and 186, and analyzing the phages transduced by the particles released from single P1*vir*- or P1*kc*-infected cells, he was able to quantitate transduction (of prophage) uncomplicated by the need for recombination. He found, first, that about 0.5% of infected cells produced particles that transduced at least one of the prophages. Of those cells that produced either lambda or 21 transducing particles (the prophages are separated by 12 min on the chromosome), about 10% also produced particles carrying the other phage. This finding suggests that processive packaging can continue for at least six headfuls. Moreover, bursts in which one of these two phages were packaged had a 5- to 10-fold increased probability of also containing phages able to transduce 186, located 30 min from the closer of the other two phages. However, since bursts (at least of P1*vir*) which

included 186 and one of the other phages did not often contain the third, even though it was located between them, Harriman concluded that the distant phages had been packaged in separate processive events and inferred that cells which produce transducing phages probably encapsidate more than one processive series. This suggests that cells which produce transducing phages are a special subpopulation of infected cells in which a significant fraction of the host chromosome is packaged into transducing particles. Indeed, he estimated that as much as 3% of the host DNA in these infected cultures is packaged into transducing particles.

P1 mutants able to transduce at increased frequency have been isolated on several occasions. Wall and Harriman (199) isolated a number of mutants capable of transducing, depending on the marker scored, with a 6- to 20-fold-increased frequency. These mutants produce three to five times as many transducing particles (but normal numbers of infectious particles) as do wild-type phages. The mutations cannot be complemented by coinfection with nonmutant phages, indicating that they are not due to a loss of enzyme activity. Chromosome breakdown appears to be enhanced after infection with the mutant phages, suggesting that the creation of extra ends for packaging initiation may lead to enhanced transduction. Unfortunately, these mutants have not been further characterized. P1 does encode nucleases (such as the *res* restriction nuclease) which do not normally promote transducing particle formation (60) but which, if produced earlier in infection, might do so. Two other classes of mutant P1 which transduce with increased frequency have been identified. Mutants with deletions or insertions in *darA* (76) exhibit a 10-fold-increased efficiency of transduction. It is not certain whether this phenotype is due to the absence of DarA, which protects P1 DNA from type I restriction, or due to the lack of an unidentified protein with a cotranscribed message. The third class of mutants consists of P1 *dam* pseudorevertants (178). These phage produce increased numbers of particles (compared with Dam– phage) in *dam* hosts and also produce 10 to 20 times as many transducing particles as do wild-type P1; the nature of these mutations has not been established.

P22: Evidence for Specificity

The variation in frequencies with which P22 transduces different markers is great. Schmieger (159) measured the transducing frequencies for 28 different markers and found variations of up to 2,000-fold (although that for most markers was within a 10- to 20-fold range). Since he was careful to measure both complete and abortive (see below for definition) transductants, his results are an estimate of packaging frequencies. A range in packaging frequencies of such magnitude means that different regions of the host chromosome vary considerably in the ability to be packaged. This suggests that P22 requires a specific recognition site from which to start encapsidation, even when packaging an inappropriate substrate such as the bacterial chromosome. If the numbers of recognition sites are few (as suggested by Schmieger), some parts of the chromosome would be included in only a single processive packaging series, originating from one such site. Markers from these regions would be transduced by transducing particles containing DNA of unique sequence (see above), and markers included in the final headfuls of such processive series would tend to be encapsidated at low frequency. If this supposition is correct, chromosomal deletions or insertions will change the register of packaging series which proceed through them, and frequencies of cotransduction, even of markers not cotransducible with the register-changing DNA sequence change, will be altered (Fig. 3). This prediction was verified by Chelala and Margolin (29) and later by Krajewska-Grynkiewicz and Klopotowski (94). These experimenters compared the cotransduction frequencies of markers both close to and as far as 5 to 10 min distant from the site of a deletion. Cotransduction of pairs of markers unlinked to the deletions was increased or decreased by as much as threefold. Insertions caused similar effects. These results suggest that packaging is processive for at least 10% of the chromosome's length (10 headfuls). As expected, the register was altered only if the insertion or deletion was on the donor chromosome.

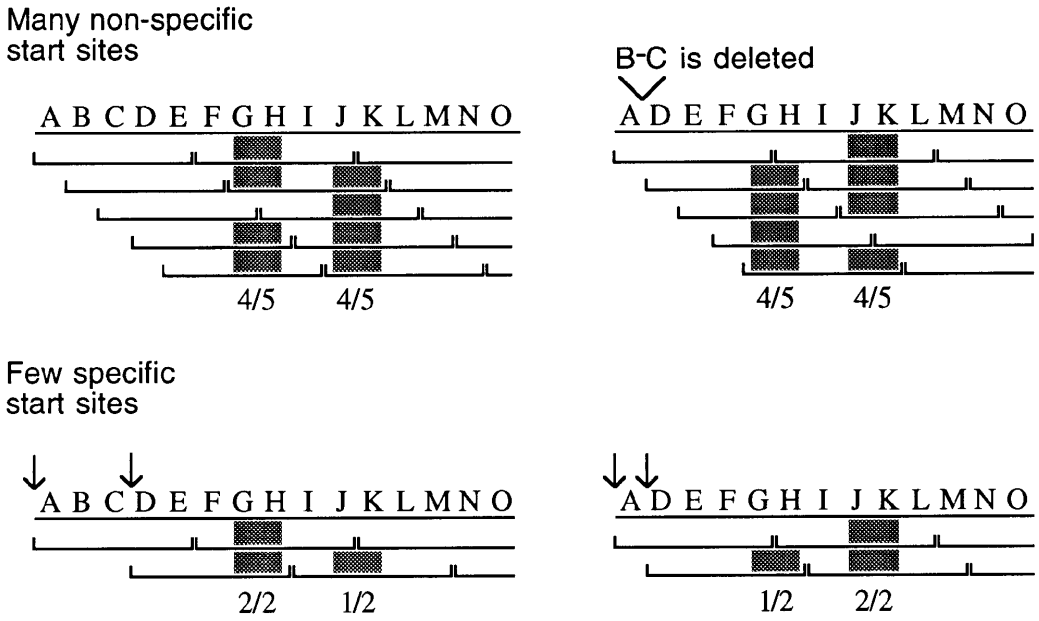

FIGURE 3 Effect of deletion and packaging specificity on contransduction frequency. The diagram shows how the deletion of B-C, which is not normally packaged with G-H and never packaged with J-K, affects the contransduction of the latter pairs of markers. The upper part illustrates the behavior of markers packaged by a phage such as P1, in which there appear to be many nonspecific start sites. Four of five molecules which include G or J will also include H or K; this is not changed by the deletion of B-C. Where there are few, but specific, start sites, as with P22, G-H and J-K, although equally linked physically, show different contransduction frequencies because they have different probabilities of being included in a single packaged DNA molecule. Deletion of B-C and change this probability giving decreased (G-H) or increased (J-K) contransduction.)

Further evidence comes from the study of high-transducing (HT) mutants. These mutants, isolated by Schmieger (158), show 100- to 1,000-fold-enhanced transduction. Transduction of markers poorly transduced by the wild-type phage is improved most; the observed range of transduction frequencies in HT lysates is thus reduced to 10- to 20-fold. This improvement in transduction results from greatly increased encapsidation of host DNA; HT phages can encapsidate up to 50% of host DNA into transducing particles. HT mutants map to gene *3* of P22 (27, 141), thought to encode one of the subunits of the packaging endonuclease, and the HT phenotype is dominant or codominant (158). These mutations might thus either alter or reduce packaging specificity or generally increase the level of the cutting which leads to transducing particle formation. One of the mutants, HT12/4, has been studied in some detail (26, 86) and has been shown to initiate the packaging of P22 DNA from two novel sites, which promote encapsidation in opposing directions. In an abbreviated cotransduction register experiment, scoring only markers linked to a deletion (30, 160), it was found that HT12/4 lysates exhibited quite different cotransduction frequencies than did P22 but remained affected by the deletion. This finding suggests that this mutant has an altered packaging specificity for chromosomal DNA as well as for phage DNA. New P22 packaging specificities were not found for the four other HT mutants studied, and for at least one of these, HT104/2, studied in some detail (94), cotransduction of a marker pair remote from a deletion was altered in the same

way as in a corresponding wild-type P22 lysate. Thus, this mutant appears to retain normal specificity for cutting bacterial DNA while cutting at increased frequency. On balance, these experiments suggest that the HT phenotype results primarily from an increased preference for encapsidating bacterial DNA rather than from a loss of specificity in selecting cutting sites, but some evidence is difficult to reconcile with this interpretation. At least one HT mutant is able to transduce plasmid DNA (R. Bauerle, *Genetics* **104**[suppl]**:**S5, 1983), which wild-type P22 does not do, suggesting an altered cutting specificity. The reduced range in transduction frequencies characteristic of HT mutants also suggests reduced packaging specificity, although increased processivity could also account for this behavior.

Introduction of *pac* into Desired Packaging Substrates

Plasmids such as pBR322 are not transduced by wild-type P22 unless they carry P22 DNA (130). If recombination is prevented by host and phage mutations, *pac* itself must be carried for successful transduction; this fact has helped in the delimitation of *pac* (154). Vogel and Schmieger (194) took advantage of this finding to seek bacterial sequences which would promote plasmid transduction. Seven distinct fragments were identified in a chromosomal DNA library but not characterized further. Unfortunately, this promising work has not been followed up.

Specific enrichment of markers in transducing lysates can be obtained by introducing *pac* into the chromosome. Kufer et al. (95) found that transduction was enhanced up to 1,000-fold for markers downstream of an integrated *pac* site (they also found, curiously, that Tn*10* appeared to have *pac*-like activity). Youderian and coworkers (213), drawing on the observation (202) that induction of a P22 prophage which is unable to excise promotes transduction of flanking chromosomal DNA, devised a powerful system able to amplify specific markers within transducing lysates to a remarkable degree. They constructed hybrid phage genomes, Mud-P22s, which contain a selectable marker and two-thirds of the P22 genome sandwiched between Mu ends. Transposition to an F′ followed by transduction and homologous recombination with resident Mu phage derivatives resulted in the insertion of these elements at different locations on the chromosome. These P22 lysogens can be induced, but as the phage are unable to excise from the chromosome, no infective phage will be produced. Phage DNA replication occurs in situ, however, and phage *pac* sites serve as the origins of packaging series which always proceed into chromosomal DNA to one side of the insertion. Markers located in the first non-P22-containing headful can be transduced with efficiencies 4 orders of magnitude greater than usual (particles containing them comprise the majority of particles in the lysate), and markers 6 min away can be transduced with 1,000-fold-increased efficiency. Benson and Goldman (10) used these materials to construct a set of insertions at approximately 3-min intervals around the *S. typhimurium* chromosome. Lysates prepared from these strains can be used to map rapidly any mutation with a selectable phenotype, since effective transduction can be accomplished on plates by spotting transducing lysates on recipient cells. These lysates have been used not only for mapping but also to identify recombination-deficient mutants (10a).

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, and persistence within the cytoplasm in a form refractory both to recombination and degradation. For the P1 and P22 systems which we have been considering, only 1 to 2% of the transduced DNA is ordinarily recombined into the recipient chromosome.

Some Transduced DNA Is Degraded and Recycled

Transduced DNA resists degradation. Thus, successful transformation with linear DNA requires the use of nuclease-deficient hosts, whereas transduction does not. The landmark studies of Ebel-Tsipis et al. (49) for the P22-*S. typhimurium* system and of Sandri and Berger (152) for the P1-*E. coli* system demonstrated that no more than 10 to 15% of transduced DNA is degraded to its component nucleotides in wild-type cells. These workers monitored the fate of labeled transducing DNA in recipient cells for up to 5 h after transduction and found that no more than 15% of the label was incorporated into recipient DNA in a form not recoverable in the continuous patches expected for the products of recombination. Since this incorporation occurred even in recombination-deficient recipients, it was concluded that it must originate from recycled nucleotides, defining 15% as the fraction of transducing DNA liable to degradation.

The Dar internal head proteins of P1, as mentioned above (75), protect DNA that has been packaged in P1 heads from subsequent degradation by type I strain-specific restriction endonucleases in recipient cells of different *hsd* genotypes. This system, clearly of selective benefit to a phage with the wide host range characteristic of P1, protects transducing as well as virion DNA.

Abortive Transduction

About 15% of the label in transduced DNA becomes physically associated with recipient chromosomal DNA; this includes both recombined sequences (discussed below) and recycled nucleotides. The remainder of the transduced DNA, up to 90% of the total, remains within the cytoplasm in a stable form and neither replicates (101, 103) nor is degraded (49, 152). It can be physically recovered, undiminished in quantity, for at least 5 h after infection (152). It does not appear to be a substrate for recombination. Most recombination occurs during the first hour posttransduction (49); DNA that fails to recombine during this initial period rarely does so later.

This stable DNA is referred to as abortively transduced DNA, and the cells that harbor it are referred to as abortive transductants. Abortive transduction, first noted in some of the earliest reports on transduction in *S. typhimurium*, was proposed to explain the phenomenon of trailing (101, 179, 180), observed on plates used to score the inheritance of motility. When plated on semisolid agar, motile bacteria swim and form swarms rather than discrete colonies. However, most of the progeny of transductional crosses instead form trails, consisting of strings of colonies of nonmotile cells, each apparently arising from a nonmotile descendant of a single motile cell. It was suggested that the parent motile cell was an abortive transductant, harboring a stable, nonreplicating but transcriptionally active fragment of transduced DNA. Later, abortive transductants for various nutritional markers were found among the progeny of P22 (132) and P1 (58, 65) crosses. These can be observed as 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. Again, this behavior can be understood if a stable, transcriptionally active but nonreplicating DNA fragment is inherited unilinearly. Daughter cells may inherit sufficient protein product to enable them to grow and possibly even to divide once or twice, but only the original cell will be capable of the repeated divisions required to form a microcolony. Abortive transductants are only very rarely converted to complete transductants; Ozeki and Ikeda estimated a conversion rate of 0.001 per generation on the basis of their observation that only 7 of 1,000 microcolonies became fullsized after prolonged incubation (134), again suggesting that if successful transductional recombination does not occur soon after DNA entry, it is unlikely to occur at all. The fraction of total transductants which are abortive varies from marker to marker (Schmieger [159] found ratios of complete to abortive transductants of between 1:1 and 1:72), but for many markers no more than 10% of transductants are complete (134, 159), an observation consistent with the physical observations described above.

Thus, it appears likely that the bulk of P1 and P22 transduced DNA assumes the abortive configuration within the recipient cell. Once in this configuration, it is metabolically active but resists degradation and recombination; since it cannot be replicated, it is unilinearly inherited. What, then, is the abortive configuration? Experiments in which phage lysates have been irradiated before transduction have provided some clues. Increased numbers of complete transductants are recovered when lysates are irradiated before use (1, 54, 68, 110, 127, 199). This appears to be at the expense of abortive transductant formation; in experiments in which abortive transductants were counted, their numbers were reduced concomitantly with the increase in complete transduction (11, 209). In experiments with P1, it was found that transduction of markers which are normally relatively poorly transduced is specifically enhanced by UV irradiation of the donor lysates, leading to the loss of much of the marker-specific variation in transduction frequency. UV enhancement of transduction requires UvrA and UvrB (69; B. Newman, Ph.D. thesis, University of Edinburgh, Edinburgh, Scotland, 1982), enzymes involved in the excision of UV-damaged DNA sequences to create single-stranded gaps. Treatments such as X rays and ^{32}P decay, which are likely to yield nicked rather than gapped DNA, do not enhance complete transduction (66, 188). Taken together, these studies suggest that UV irradiation promotes the conversion of transducing DNA to a form which, probably as a result of gaps introduced during the process of repair, is rendered recombinogenic. Thus, we can deduce that abortively transduced DNA lacks such gaps.

But what protects the ends of abortively transduced DNA, which should be prime targets both for nuclease action and for recombination enzyme entry? The most likely candidate is a protective protein, possibly that reported by Ikeda and Tomizawa to be associated with the ends of transducing, but not of P1 phage, DNA (79). Sandri and Berger (152) investigated this putative association further by extracting density-labeled, abortively transduced DNA from recipient cells and seeking an association of this DNA with protein. They found that abortively transduced DNA migrated unusually quickly in velocity gradients but that treatment with pronase or detergents decreased its rate of sedimentation to that of linear phage DNA. Supercoiled circular DNA sediments more quickly than linear DNA; gel analysis verified that abortively transduced DNA migrates as supercoiled DNA. Treatment with pronase or detergent appeared to convert the abortive DNA to a species which migrates through gels as does linear DNA. These results suggest that abortively transduced DNA assumes a circular supercoiled configuration within recipient cells but that the DNA circles are not covalently closed. Rather, a protein or proteins hold together the ends of the originally linear DNA, making it resistant to both recombination and degradation.

What might this protein be, and where does it originate? If it is the protein reported by Ikeda and Tomizawa to be associated with transducing DNA, then it must originate in the donor cell and be packaged along with the DNA. If it is a P1-specified protein, as seems likely, then why is it associated with transducing but not with infective DNA (see above)? Yarmolinsky and Sternberg (212) suggest that the protecting protein is in fact the packaging endonuclease. They hypothesize that when bound to a genuine *pac* site, the protein undergoes a change which causes it to be processively transferred along a concatemeric packaging substrate, initiating headful cuts as appropriate. If it is initially bound to a bacterial DNA substrate, possibly at a free end, the enzyme might instead remain associated with that end and a new molecule may be required for each successive cut. The bound molecules would then be packaged along with the DNA. Once in the recipient cell, the protein might associate with the other end of its former DNA substrate, leading to the protective circularization observed. If this were the case, then it might be expected that when transducing particles originate from a chromosomally integrated *pac* site, no abortive transductants will result. This prediction should not be difficult to test.

Schmieger found that the DNA from P22 transducing particles prepared on density-labeled hosts had a lower density than did parental DNA (158). He could not, however, find any evidence that protein binding was responsible for density reduction, leaving open the question of how abortive transductants of P22 might be stabilized. Happily, there is new information relating to this question. Benson and Roth (10a) find that one of the P22 HT mutants, HT12/4, actually harbors two mutations, one of which results in the failure of this phage to yield abortive transductants. This mutation can be separated from that in the endonuclease

and maps to gene *16*, an essential gene whose product is packaged in the phage head and is thought to be injected into the recipient cell along with the DNA. These authors suggest that gp16 is an end-protective protein and is responsible both for the stability of abortive transductants and for the survival of injected phage DNA until it is circularized by recombination. In the light of this information, the existing physical data require reassessment. Unless gp16 is not attached to DNA until the time of injection, the densities of both phage and transducing DNA should be lowered as a consequence of association with the protein. If this view of gp16 function is correct, it appears likely that the origins of the proteins which lead to stabilization of abortive transductants are quite different for P22 and P1.

Finally, several mutations in genes encoding internal P1 head proteins require mention in the context of abortive transduction. DarA and DarB (75), also described above, are injected antirestriction proteins. In transduction between strains with the same *hsd* genotype, *dar* mutants show up to 10-fold-increased stable transduction and correspondingly decreased abortive transduction, suggesting that Dar normally affords protection against other nucleases in addition to the type I restriction endonucleases. The Dar proteins are found in the heads of both infectious and transducing particles; they cannot therefore be identical to the transducing-DNA-associated protein of Ikeda and Tomizawa. *sus50* mutants (209), defective in tail attachment, also show decreased abortive transduction and infectivity; stable transductants per infective unit or per abortive transductant are increased 100-fold, but the number of transductants per particle is unchanged. Thus, the *sus50* mutant encapsidates normal (or perhaps even reduced) amounts of transducing DNA which, however, fails to achieve the abortive configuration. Whether this is because it is damaged and hence more recombinogenic or because it lacks the end-protective protein has not been established. Since *sus50* mutant phages contain the Dar proteins, they must be affected in a different gene. Whether Dar or the *sus50* gene product has a role in the circularization of abortively transduced DNA is unknown; however, the fact that the Dar proteins have been shown to protect associated DNA from nuclease action is sufficient to explain why their absence might potentiate recombination.

Stable Transduction

Ebel-Tsipis et al. (49), for P22, and Sandri and Berger (152), for P1, traced the fate of the labeled donor transducing DNA which becomes associated with recipient chromosomal DNA. Although the two studies were carried out a decade apart, with different systems and in different laboratories, their results are comparable. Most of the chromosome-associated DNA is evenly distributed along the chromosomes of either Rec^+ or Rec^- recipients and represents recycled nucleotides, as described above. The remainder, amounting to about 2% of the total transduced DNA (and 15 to 20% of the integrated DNA), can be recovered, but from Rec^+ recipient DNA only, in continuous stretches 500 or more bp in length. These patches are the putative products of recombination.

Physical analysis was performed on this DNA in an effort to determine its length and whether incorporation is of single or double strands. To do this, chromosomal DNA was purified from transduced cells about 30 min posttransduction, and the average size of fragments was decreased by shearing, endonuclease action, or sonication. Unsheared DNA contained a shoulder of heavier DNA when separated by density on CsCl. When the DNA was fragmented to about 10 kb in length, part of the shoulder DNA was converted to DNA that migrated with the fully heavy donor DNA, suggesting that double-stranded donor fragments of about this size were among the primary products of recombination. When time was allowed for replication before sampling, these double-stranded donor fragments were replaced by DNA of hybrid density, as would be expected. The fact that not all of the shoulder DNA in the early samples, at least in the case of P22, was convertible to heavy DNA suggests that there may well also be single-strand in addition to double-strand integration.

Integration of fragments approximately 10 kb in length cannot account for the observed frequencies of cotransduction observed for P1 (see Fig. 5). Ten kilobases represents approximately 0.2 min on the *E. coli* chromosome; markers that close are 80% cotransduced, while those separated by 0.5 min are 50%

cotransduced. Thus, at least half of all integration events ought to incorporate stretches of at least 20 kb. It should be noted that in Sandri and Berger's experiments, in which transductional integration was analyzed in detail, P1 lysates were irradiated to increase recombination levels (two- to fourfold). The resulting damage to the DNA might well have led to the integration of shorter than normal fragments. Support for this idea comes from experiments done by Milkman and Bridges (124) in the course of an investigation into the mechanism of bacterial molecular evolution. These authors sequenced the *trp* regions of a series of distantly related *E. coli* strains and observed that in many strains, the region appears to be composed of a mosaic of fragments differing in clonal origin. A possible route by which such mosaics could have arisen, supported by the analysis of the products of P1-mediated interstrain crosses using *E. coli* K-12 as the recipient (121a), is through multiple recombinational events between a single exogenote and the chromosome. Backcrosses to K-12 recipients, however, show the inheritance of much longer continuous sequences, suggesting that the results obtained from interstrain crosses are more likely to be due to incompatible restriction systems than to be inherent in the mechanism of transductional recombination. Recent work by Miesel and Roth (123), in which RecBCD exonuclease-deficient *S. typhimurium* cells were transduced with P22 at low multiplicity, shows that the successful transduction of long fragments is greatly enhanced in these mutants. P22 transductions are often carried out at high multiplicity; in such crosses, a phage-encoded inhibitor of RecBCD action is expressed (139) and permits the integration of long fragments of DNA. In the experiments of Ebel-Tsipis et al. (49), purified transducing phages, free of infectious phage and consequently lacking RecBCD inhibitor, were used. This could explain why these experimenters found that the integrated DNA fragments were short. Further reexaminations of posttransductional integration events using current methodology and information would be welcome.

It has been noted many times that numbers of transduced progeny fail to increase for up to several hours after transducing phage addition (8, 49, 59, 152). Hanks and Masters (59) showed that for *E. coli* transduced with P1, the length of this delay is related to the chromosomal position of the marker assayed; transductants for markers near the terminus of replication increase in number first, and those near the origin do so last. These results can most easily be understood if transductional recombination is primarily the product of single-strand replacements; completion of two rounds of replication and division would then be required to produce two progeny cells with the transduced phenotype. This would occur earliest for those markers nearest the replication terminus.

Pathways of Transductional Recombination

The numbers of P1 transductants obtained for particular markers vary with the concentration of those markers in the donor cell (21, 116). If the transducing ability of lysates prepared from cells with multiforked chromosomes is compared with that from cells in which DNA replication occurs at a single fork, it is possible to deduce the order of replication of the markers tested; this technique has been used to locate approximately the origin of chromosome replication (119). If, instead of making such a comparison, the frequency of transductants obtained from a single lysate is examined, there is considerable variation between markers superimposed upon the prominent gradient from origin to terminus. Since the observed degree of variation is not attributable to differences in packaging (117), it must be a consequence of the way in which recombination occurs in the recipient cell.

Most recombination in wild-type cells of *E. coli* (and probably also in those of *S. typhimurium*) is thought to proceed via the RecBCD pathway (for reviews, see references 33, 34, 167, 168, and 203, also chapter 119 of this volume). RecBCD-mediated recombination is believed to be initiated when the RecBCD enzyme invades flush-ended double-stranded DNA and separates the strands, with concomitant degradation of the 3′ strand. This degradation is thought to cease when the recombinogenic sequence *chi*, in the correct orientation, is encountered (40). The product of RecBCD action, now with a single-stranded tail originating near *chi*, is thought to invade the double helix to form recombinational intermediates. This succession of events favors crossovers in the vicinity of *chi*. If the same process were to occur at each end

of the transducing fragment, a primarily double-stranded section of donor DNA would replace an equivalent length of recipient DNA. The length of the integrated fragment should vary with the position of *chi* relative to the ends of the fragment, but if *chi* occurs once per 5 kb, as appears to be the case, the average length of integrated fragments should not be much less than the length of P1. This is not in accordance with the physical observations described above but is reasonably consistent with cotransduction frequencies. If *chi* were oriented preferentially in one direction on the chromosome, the average length (although not the distribution in lengths) of integrated fragments could be much less. If RecBCD were active at one end of the transduced fragment only, perhaps because the other end is protein protected, the formation of viable recombinants could require multiple events.

Evidence documenting crossovers in the vicinity of *chi* during transductional recombination comes from several sources. Dower and Stahl (41) carried out transductional crosses between lambda lysogens in which the integrated phages were Chi^+ or Chi^-. They found that crossover frequency in the vicinity of *chi* is enhanced in a RecB-dependent manner, especially when *chi,* in the correct orientation, is present in the donor sequence. *recD* mutants are nuclease deficient and *chi* independent for recombination. Lloyd et al. (108) found that recovery of transductants for a marker which had previously been poorly transduced (*car*) was enhanced in *recD* mutants, while that for a neighboring marker normally transduced at high frequency (*leu*) was unaffected. This finding suggests that *car,* possibly because of its position vis-à-vis a *chi* site or sites, is particularly subject to RecBCD degradation. It would be interesting to determine whether abolition of RecD activity would systematically reduce recombinationally caused variations in transduction frequency. The work of Miesel and Roth (123), described above, implicates RecD, and by inference *chi,* in P22-mediated transductional recombination in *S. typhimurium.* They suggest that RecBCD nuclease, if not inhibited, limits the size of the incorporated fragment to about 20%, on average, of the length of the encapsidated DNA. This would be about 10 kb in length, in agreement with the findings of Ebel-Tsipis et al. (49).

Recombinational discrimination can be eliminated by altering the recombination pathway. The RecF pathway, probably responsible for a maximum of 10% of transductional recombination in wild-type *E. coli* cells (34, 120), becomes an efficient pathway for recombination in *recBC sbcBC* cells. We showed (120) that under these conditions, transduction of poorly transduced markers is greatly enhanced, again suggesting that discrimination by RecBCD is a determinant of transduction frequency. In *recBC sbcBC S. typhimurium,* abortive transduction levels are reduced (Benson and Roth, personal communication), indicating that the enhancement in complete transduction characteristic of RecF-mediated recombination is likely to be at the expense of abortive transduction. RecF-mediated transductional recombination also leads to the integration of longer fragments than does RecBCD recombination (36, 123; M. Masters, unpublished results), possibly because of the reduced degradation of linear DNA molecules (35) consequent upon the reduced nuclease activity required for efficient RecF-mediated recombination. The molecular details by which the RecF pathway initiates recombination remain uncertain, but it will be interesting to discover how, in these nuclease-deficient cells, undamaged, abortively transduced DNA is efficiently converted into a recombinational substrate.

Recombinational discrimination can also be eliminated (120) and recombination frequency can be enhanced (at the expense of abortive transductants) by UV irradiating transducing phage before infection of the host (1, 11, 54, 68, 110, 127, 199, 209). Enhancement does not require *recB* function (120), suggesting that the DNA damaged by UV does not primarily recombine via the RecBCD pathway. The RecF pathway has been reported to operate more efficiently (33, 90, 109; chapter 119 of this volume; Masters, unpublished results) in SOS-induced cells (several genes whose products are required for RecF pathway recombination belong to the SOS regulon [107]), and as the damage sustained by irradiated transducing DNA could well induce SOS, it is possible that the RecF pathway is responsible for the recombination of UV-damaged DNA. This is consistent with the observation that the action of RecBCD is confined to linear double-stranded substrates; the gapped circular molecules created by irradiation and partial repair of what would have been abortively transduced DNA molecules are

necessarily substrates of a recombinational system other than RecBCD.

Markers linked to *oriC* are transduced with unexpectedly high frequencies, at least fourfold higher than that of neighboring markers (117). This enhanced transduction is not due to selective packaging (60) and is associated with an absence of abortive transduction. Furthermore, all progeny of even the earliest divisions, those occurring before chromosomes containing DNA integrated near *oriC* can have completed replication and separated, can inherit *oriC*-linked markers (Masters, unpublished results). This can be most easily understood if transducing fragments containing *oriC* are able, even as exogenotes, to initiate replication. This would provide multiple copies of *oriC* DNA (probably in the form of single abortive molecules with partially replicated DNA) as substrates for recombination. A copy of the *oriC*-linked sequences would thus be available to recombine with each *oriC* copy in the cell. Enhanced recombination has also been noted for markers linked to plasmids integrated in the chromosomes of donor DNA (Masters, unpublished results), consistent with this interpretation.

Figure 4 summarizes some of this information and presents a model describing the possible fates of transducing DNA in the recipient cell.

OTHER GENERALIZED TRANSDUCING PHAGES

A variety of other phages, in addition to P1 and P22, can carry out generalized transduction. Margolin (114) and Sternberg and Maurer (178) have described some of these in considerable detail. Unfortunately, there have been few developments in the understanding of these systems since the publication of these earlier reviews. Table 1 lists some of these phages and their salient properties.

P1 and P22 have in common the facts that they are temperate and that they package phage DNA by sequential headful packaging from concatemers starting near a specific packaging recognition site, *pac*. Encapsidation of transducing DNA occurs from chromosomal sites, possibly sites which resemble *pac*, DNA ends, or both. We thus might expect that other phages which package DNA by the headful could also serve as vectors for generalized transduction. Coliphages T1 (56) and T4 (183) package DNA in this way, but since successful transduction requires both that donor DNA not be degraded before it is packaged and that recipient cells avoid lysis by superinfecting phages, virulent phages would not be expected to be useful transductional vectors. However, although transduction does not normally accompany infection with T1 or T4, systems that use mutant phages and hosts and that circumvent these difficulties have been developed for both phages; these are described below.

The temperate bacteriophage Mu is also thought to package DNA by the headful, and Mu, like P1 and P22, is able, without modification, to carry out generalized transduction (72). Transduction by the *Salmonella* phages ES18 (97), which has some affinities with P22, and KB1 (16, 121), which does not, was described briefly by Margolin (114). These latter phages do not appear to be in current use, and I will not discuss them further here.

Finally, there is lambda. Since lambda utilizes a site-specific cutting mechanism to package its DNA, albeit from concatemers, it would not be expected to be a vector for generalized transduction. Despite this, an efficient transduction system has been developed, although both the use of a mutant vector and the use of an in vitro maturation step are required (171).

T1

T1 virion DNA is about 47 kb long (112). Heteroduplex analysis was used (56) to show that T1 packages its DNA by the headful; three classes of DNA, permuted by 6%, predominate within infectious particles. Packaging begins at a site between genes *1* and *2*, seldom proceeds for more than four headfuls, and after the initial cut, is sequence independent (111, 142). Concatemers greater than four times unit genome length were not found among phage DNA precursors (144), suggesting a probable reason for the limited permutation characteristic of T1. T1 development and, briefly, transduction have been reviewed elsewhere

(45).

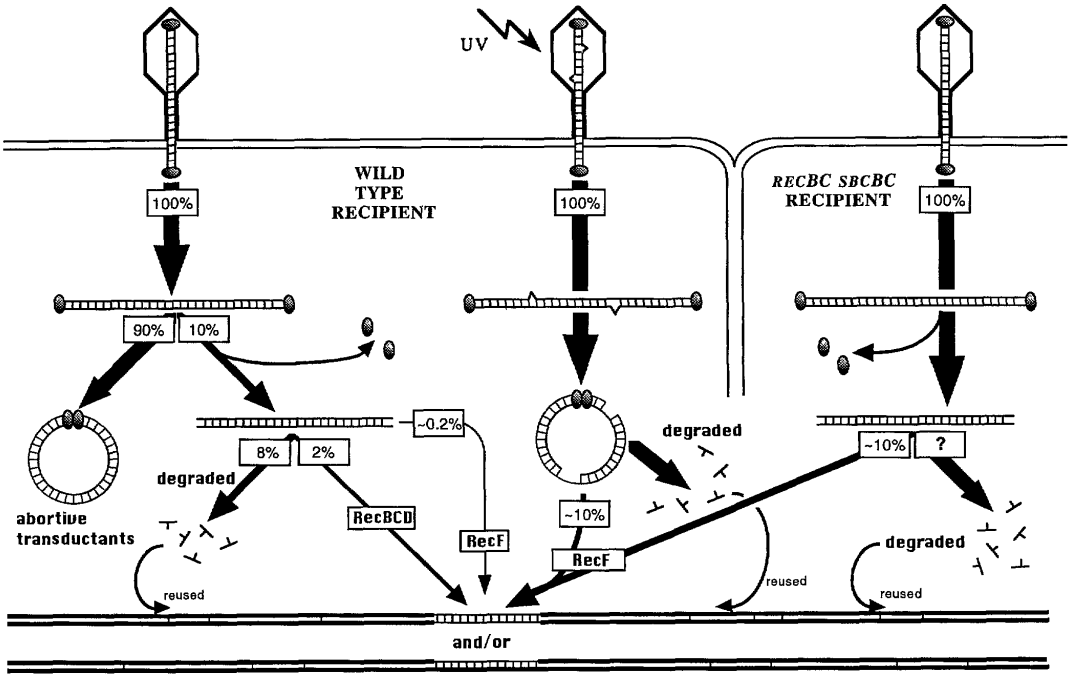

FIGURE 4 Fate of P1-transduced DNA in the recipient cell. The figure shows intact or UV-damaged double-stranded transducing DNA, with ends protected by protein, after entry into a wild-type or *recBC sbcBC* recipient. Widths of arrows and the numbers in the boxes indicate the fraction of incoming DNA with the indicated fate. Text in boxes shows the recombinational pathway believed to be active in each case. The double lines at the bottom represent recipient DNA which has undergone recombination by double- or single-strand replacement.

TABLE 1 Transducing phages and their properties[a]

Phage	DNA content (kb)	Stable transductant frequency (reference)	Special conditions, comments
P1	100		
P1*kc*		10^{-4}–10^{-5}/PFU (120)	Low MOI to avoid lysogenization
P1*vir*^{s}		10^{-5}–10^{-6}/PFU (199)	Low MOI to avoid killing
P1Cm*c*1.100		10^{-5}–10^{-6}/AP (147)	Lysates by induction
P22	44	10^{-6}–10^{-9}/PFU (158)	*int* phages used to avoid lysogenization at high MOI
P22HT		10^{-4}–10^{-5}/PFU	
T1	47	10^{-6}–10^{-8}/SIC (42)	Must use T1*amb*, nonsuppressing recipient, and low MOI
T4 GT7	172	10^{-5}–10^{-7}/AP (204, 214) ~10^{-4}/SIC	Low MOI required; lysates are very stable
λ *s red*	48.5	10^{-5}–10^{-8}/donor cell (176) (0.001% of P1 yield)	In vitro treatment of lysate
Mu	40	10^{-7}–10^{-8}/PFU (72)	Mini-Muduction increases frequency 10–50X

[a]AP, adsorbed phage; MOI, multiplicity of infection; SIC, singly infected cell.

By growing a T1 amber mutant on a donor strain and using a nonpermissive host as a recipient, Drexler (42) was able to use T1 as a transductional vector. The vector phage, although unable to propagate on the recipient, can still kill it, and thus transduction must be performed at low multiplicities. Transduction frequencies, measured per SIC, are low and vary greatly with the marker assayed; for five markers measured (these do not include markers linked to *oriC;* see above), frequencies of between 10^{-6} and 10^{-8} per SIC were found. This observation suggests that transducing DNA is packaged selectively rather than at random, paralleling the behavior of P22 rather than that of P1. Consistent with this supposition is the fact that plaque formation by transduced Mu prophage, used as a measure of packaging because it does not require recombination at any specific site, also varies over a wide range dependent upon its chromosomal location in the donor cell (9). It is not known how many sequential headfuls of chromosomal DNA are likely to be packaged into transducing phages or whether encapsidation starts at sites that resemble T1 *pac*.

T1 derives most (60 to 70%) of its nucleotides from degraded host DNA (99), suggesting that transducing DNA must be packaged early. This does appear to be the case (98); transducing phage are two to five times enriched in premature lysates, and premature lysates which contain only 25% of infectious particles already contain 50 to 90% of transducing particles. Roberts and Drexler (145, 146), by screening for the transduction of lambda to lawns of mixed indicators which included T1-resistant, lambda-sensitive hosts, succeeded in isolating mutants of T1 able to transduce at increased frequency. Each mutant proved defective in host DNA degradation, leading to decreased burst size but increased ability to transduce; frequencies of 10^{-3} to 10^{-4} per SIC were obtained.

The *bio* gene is transduced at a higher frequency than were other markers measured. This appears to be due to the proximity of a chromosomal site (designated *esp*) hypothesized to be a *pac*-like sequence (43). Lambda is also thought to contain such a sequence (44). pBR322 does not; it is transduced at extremely low frequency, although inclusion of lambda *esp* can increase this frequency 100-fold. The inclusion of *pac* in a similar plasmid increases plasmid packaging frequency remarkably (104). Lysates in which 10% of particles contain plasmid DNA can be obtained, with the packaged form most likely a head-to-tail plasmid multimer. Resolution of these multimers in the recipient cells to yield transductants seems inefficient, as plasmid transduction frequencies are only 10^{-3} per SIC. Plasmids containing other T1 sequences can also be transduced at elevated levels (10–5 per SIC), but their establishment in the recipient requires a T1 recombination function, suggesting that the transduced forms are T1-plasmid cointegrates. Successful transduction of all plasmid forms requires that the recipient be recombination proficient.

Irradiation of T1 lysates does not increase transduction frequency (46), indicating that abortive transductants are not formed and lending credence to the supposition that abortive transductants owe their genesis to adaptations of P1 and P22 which promote lysogenization of their hosts.

T4

Since T4 packages its DNA by the headful, virion DNA is terminally redundant and circularly permuted (182, 183, 205). Mature T4 DNA is 172 kb long; thus, the capacity of a T4 transducing phage would be 3.5 min of chromosomal DNA. This would permit the cotransduction of markers too far apart to be cotransduced by P1, possibly advantageous in some circumstances. Wild-type T4 does not transduce, in part because host DNA is destroyed early in infection. However, Wilson et al. (204) found that a derivative of T4, which had been developed for molecular cloning, can serve as a transductional vector. This phage, T4GT7 (hereafter referred to as T4), has a number of mutations which either are essential for or strongly contribute to its ability to transduce. These include mutations in *ndd* (host nucleus disruption), *denB* (endonuclease IV, specific for cytosine-containing DNA—T4 DNA contains glucosylated-OH methylcytosine rather than cytosine and synthesizes this precursor from degraded host DNA), dCTPase, deoxyhemimethylase, and *alc* (allows late transcription on cytosine DNA), all of which contribute to extended survival of host DNA. A deletion of the D1 region and an *r*II A or B mutation are also important, but the mechanism by which they assist is not known (215). If the bacterial host is labeled before infection,

transducing phage particles can be separated from infectious by density, indicating that they carry host DNA made before infection (214).

Transduction is achieved by infecting recipients at a low multiplicity of infection. Frequencies of 10^{-4} per SIC (204), or 10^{-5} to $^{10-7}$ per adsorbed phage (214), were obtained and span broadly the same range of frequencies as are obtained with P1, with a weak correlation between the values obtained with the two phages. Among 29 markers tested, 4 which were transduced by P1 in parallel experiments were not transduced by T4 (214). However, later work (137) showed that two of these could be transduced, suggesting that T4 can probably transduce any marker given the appropriate conditions. It has been noted (137) that T4 is particularly useful for transduction from *recA* donors, as P1 grows poorly on *recA* strains. This work also measured cotransduction between *recA* and two moderately distant markers: *cysC,* 1.25 min distant in the donor used, cotransduced at 10%, and *thyA,* 2.9 min distant, cotransduced at 4%. Although these results indicate that T4 use does indeed provide a valuble extension to the length of DNA that can be transduced between *E. coli* strains, they also suggest that markers further apart than 3.0 min probably cannot be usefully cotransduced.

Several studies report transduction of plasmids by T4 (89, 128, 186, 187, 204) or T2 (32). Transduction can be efficiently obtained (1 of 50 surviving cells can inherit plasmid) and appears to be mediated by particles whose DNA consists of head-to-tail concatemers (186). These are probably formed by replication after infection (128). Since T4 lysates remain stable for many years, it has been suggested (187) that the phage could be used as efficient containers for storing plasmid DNA!

Lambda

Generalized transduction by lambda was first reported by Sternberg and Weisberg in 1975 (177) and later described in more detail (171). The process has also been summarized, with methods (178). The generation of lambda generalized transducing phages requires two mutations in the phage. Because transducing particles appear to be formed only very late in infection (between 60 and 120 min after induction or phage addition), a mutation in the *S* gene, which delays lysis, is essential. The second mutation required is a *red* mutation which inactivates lambda exonuclease, suggesting that the presumptive packaging substrate, possibly free DNA ends, is destroyed by exonuclease action.

Lambda packages its DNA unidirectionally from a concatemeric substrate; site-specific cutting at the *cos* site both initiates and terminates packaging and determines the length of the DNA packaged. Bacterial DNA lacks *cos* sites, and the mechanism by which it is packaged into lambda heads is unknown. However, the length of the DNA packaged appears to be variable, and it is often too long to fully fit into the head. This results in filled heads with protruding DNA to which tails will not attach. This problem can be simply corrected in vitro by addition of DNase to the lysate; any protruding DNA is digested, and tails, also liberated at cell lysis, then attach spontaneously.

In lysates made by infection, the transduction frequency is not high, varying between 10^{-6} and 10^{-5} per donor cell (about 100-fold lower than for P1). Encapsidation is probably much higher than these figures suggest, since only about 5% of phage particles matured in vitro can successfully inject their DNA. In lysates prepared by induction, transduction for markers within 10 min of a replication-proficient lambda prophage is increased, up to 200-fold for markers nearest the attachment site. This is almost certainly due to in situ bidirectional replications of the integrated phage which extend into adjacent bacterial DNA. Since this enhancement is not confined to excision-deficient prophage, such replication seems to be a normal concomitant of the induction process. Quantitative Southern hybridization studies show that lambda-induced replication of prophage-proximal DNA is only 20-fold rather than 200-fold, suggesting that this DNA is a favored substrate for encapsidation. This is not solely because of unidirectional packaging starting at *cos* because transduction to both sides of the prophage is enhanced. Abortive transductants have not been sought or demonstrated, although the mechanism by which transducing particles are matured suggests that they are not likely to occur.

Mu

If P1 can be considered to be a plasmid which, in an alternate guise as a phage, can be transmitted between cells, Mu can be classified as a transposon cum phage. The Mu virion contains a linear DNA molecule composed of the 37-kb phage genome flanked by a 50- to 150-bp segment of host DNA at the left end and a 0.5- to 3-kb segment of host DNA at the right end. Immediately after injection, this DNA can be recovered in a circular form in which the ends are held together by the phage N protein which is injected along with the DNA. This form is short-lived; the Mu genome, but not the terminal host sequences, quickly becomes inserted into the chromosome of the new host and, during a productive infection, proceeds to replicate by a transpositional mechanism. The eventual product of replication is thought to be a host chromosome containing about 100 noncontiguous copies of Mu scattered throughout its length. The packaging substrate may be either this long molecule or some subportion of it generated during transpositional replication. Packaging is deduced to be by a headful mechanism, as insertions or deletions of sequences into or from Mu genomic DNA lead to compensating changes in the length of the long terminal host sequence. Packaging initiation requires the integrity of a *pac* site located near the left-hand end of Mu. This information is gleaned from a monograph (185) and from comprehensive reviews (62, 133).

Howe, in 1973, reported (72) that Mu can carry out generalized transduction. Although it cannot be excluded that some of this transduction is attributable to the host sequences at the right-hand end of Mu, the fact that cotransduction occurs at the approximate frequencies that would be expected for a transducing fragment the size of Mu suggests that generalized transduction is primarily due to the encapsidation of bacterial DNA fragments. Transduction frequency is low, occurring at 10^{-7} to 10^{-8} per PFU for most markers; *leu* and neighboring markers are transduced at higher frequencies, 5- to 20-fold greater than average, depending on strain (6). The reason for enhanced *leu* transduction is not known, but it is interesting to speculate that it might be related to the preferential transduction of *leu* by P1.

If bacterial DNA is density labeled before infection, Mu transducing particles can be separated from infectious particles on the basis of density. They contain bacterial DNA of hybrid density and are composed of one strand made before and one strand made after infection (189). Preexisting DNA can, however, also be packaged (191). The initial structure assumed by injected virion DNA suggests that abortive transduction would be common, and abortive transductants, at 100 to 1,000 times the stable transductant frequency, are indeed found (72). Surprisingly, UV irradiation increases stable transduction frequency only five- to sevenfold; perhaps some of the abortive transduction arises not from circularized full-length transducing DNA but from expression of the long terminal repeat in infectious particles destined for lysogeny.

It is not clear whether transducing particles arise from separate packaging initiations at *pac*-like sites or as a result of processive packaging following on from the packaging of virion DNA, as suggested by Schroeder et al. (162). Mutants of Mu with increased ability to transduce have been isolated (190); some of these transduce only the *leu* region with increased frequency, and the remainder transduce all markers at enhanced frequency. These mutants have not been analyzed further.

Generalized transduction can also be carried out by using Mu derivatives (mini-Mu) with reduced genome size (52). Mini-Mu elements, containing only the terminal regions and gene *A*, can, when induced in the presence of a helper, be packaged along with 30 kb of adjacent host DNA. The resulting lysates transduce at frequencies enhanced 10- to 50-fold. Ninety percent of transductions appear to result from homologous replacement, and the remainder appear to occur by a process dubbed mini-Muduction. Mini-Muduction is a *recA*-independent addition to the chromosome of host material sandwiched between a pair of mini-Mu elements. It requires functional Mu gene *A* product and is presumed to represent a transpositional event. Mini-Muductants are not stable in $RecA^+$ recipients.

USES OF TRANSDUCTION

Transduction has been invaluable in two areas of bacterial molecular biology: genetic mapping and strain construction. Although the comprehensive, large-scale genetic maps of *E. coli* and *S. typhimurium* have been derived principally from conjugational time-of-entry studies, the ordering of neighboring genes or of mutated sites within genes has relied on transductional crosses. This is because the small size of a transduced fragment relative to the length of the chromosome can be exploited to restrict recombinational events to a short region of interest. For similar reasons, transductional methods are favored for strain constructions. Because the introduced fragment is short, transduced cells remain isogenic with their parents in all but the selected region.

Transduction has classically been used for fine-scale mapping of mutations which have been roughly mapped by conjugational or other methods. The availability of the Benson and Goldman set (see above and reference 10) of Mud-P22 prophages provides a way to use transduction for the initial mapping of mutations which can be expected to be be widely applied for *S. typhimurium.*

Transductional Mapping

Transductional mapping was thoroughly covered in the first edition of this book (114) and is also considered in chapter 137 of this volume. Applications of transductional mapping vary from relatively crude measurements of linkage calculated from frequencies of cotransduction to the ordering of mutations within genes through the use of multifactorial crosses with reciprocal sets of alleles in donor and recipient strains. Kemper (91) (for P22) and Wu (206) (for P1) derived formulae relating cotransduction frequency to physical distance on the chromosome. These formulae were based on the assumptions that (i) transducing DNA is packaged at random from the donor bacterial chromosome and (ii) inheritance in the recipient results mainly from a single pair of recombinational exchanges equally likely to occur at any point on the introduced sequences. Combining these assumptions with the estimated DNA headful content of the phage (in minutes) led to two formulae, that of Kemper, $F = 1 - t + (t \times \ln t)$, and that devised by Wu, $F = (1 - d/L)^3$, where F is the cotransduction frequency, t is the linear distance between genes measured as a fraction of the length of the DNA transduced, d is the distance between markers in minutes, and L is the length of the transduced fragment in minutes. The use and relative merits of the formulae are discussed by Sanderson and Hurley (149) and Sanderson and Roth (151). The graph of Wu based on his formula and modified for reproduction here to take account of current information regarding chromosome length and phage DNA content (Fig. 5), remains extremely serviceable, although we now know that the assumptions on which it was based are only approximations. Although for P1 assumption (i) (above) is a good approximation, the role of *chi* in promoting recombinational exchanges (41) is likely to account for the fact that many pairs of linked markers are not transduced with the same frequencies. It is advisable, when estimating distance from cotransductional frequency, to verify, insofar as is possible, that reciprocal cotransduction frequencies agree; otherwise, estimates of distances between markers are likely to be incorrect (117). For P22, assumption (i), that DNA is packaged at random, is not correct. Fortunately, P22 HT mutants transduce markers with more nearly equal frequency (158), allowing a correlation between cotranductional linkage and physical distance to be made.

Gene order can more safely be derived from multifactorial crosses in which markers are ordered on the assumption that since two crossovers are commoner than four, rare recombinant classes are likely to represent events requiring multiple exchanges. Some classical examples of detailed analyses using these strategies are for the *S. typhimurium trp, his* (7, 37, 39, 63), and *leu* (19, 113) genes and for the *E. coli trp* (210) and *ara* (58) operons. Despite the successes of these methods, anomalies which lead to the deduction of an incorrect marker order can arise (14). Transductional deletion mapping has helped to resolve some of these difficulties (15, 20, 65, 88, 115). In this type of mapping, a nonreverting deletion mutant is infected with lysates prepared on putative point mutants. If recombinants are obtained, the point mutation must lie outside the deletion; if none are obtained, then the point mutation is likely to map within it. Complementation by the exogenote resulting from

abortive transduction has also been used in cistron mapping of genes within an operon (38, 64). Abortive transductants are observable when the mutations in donor and recipient are in different genes but not when they are in the same gene.

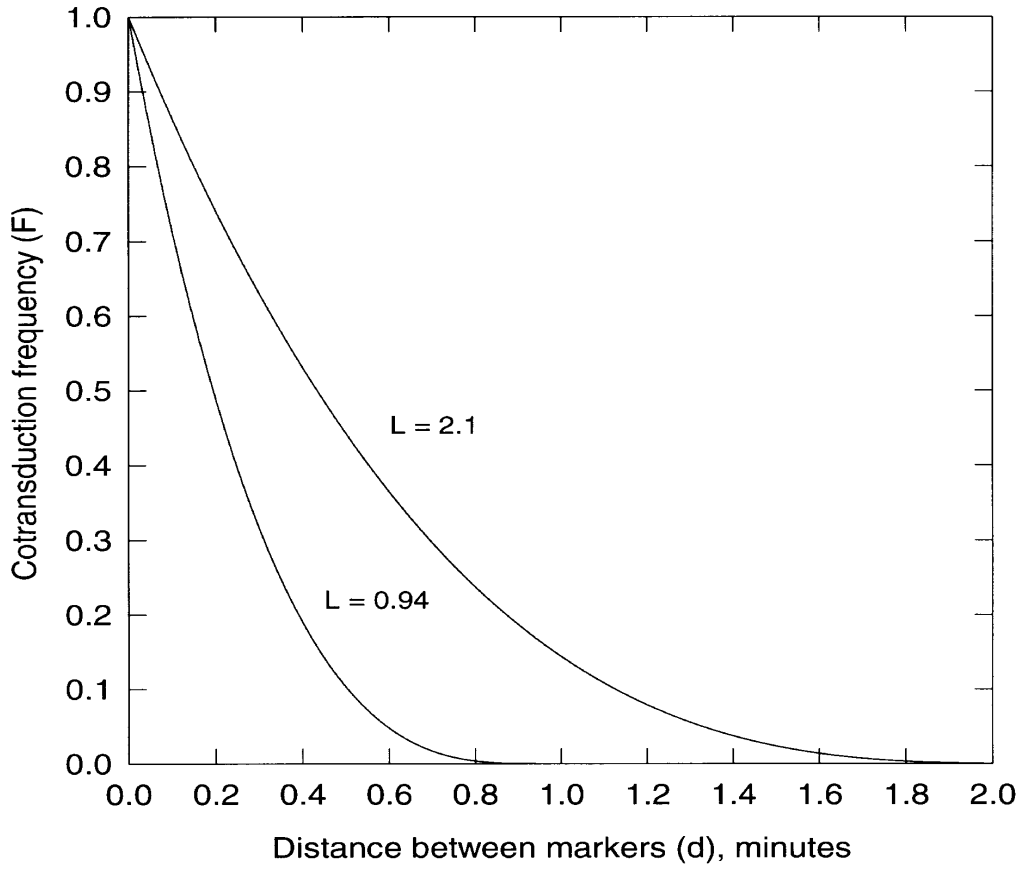

FIGURE 5 Mapping by transduction. Cotransduction frequencies (F) are plotted as a function of the distance between markers (d) according to the formula: $F=(1 - d/L)^3$ derived by Wu (206). The constant L represents the length, in map minutes, of the packaged DNA. For P1, this is currently estimated at 2.1 min. For P22, L = 0.94 min; this curve should be applicable to transductions with P22 HT mutants.

It should be noted that fine-structure genetic mapping in *E. coli* and *S. typhimurium* by using these classical techniques is not likely to be done again. The molecular technologies of cloning, sequencing, and controlled expression have become the methods of choice for defining and ordering coding units. However, transduction and cotransduction will still be used for defining the approximate locations of genes, and mutations will still be needed for defining their functions.

Constructing Strains

Localized Mutagenesis. Evaluation of the cellular role of specific gene products remains dependent on the characterization of mutants. If mutants are produced through the action of mutagenic agents on whole cells, it is difficult to be certain that additional, unwanted mutations are not generated at the same time as are the mutations of interest. Unlinked adventitious mutations can be avoided by introducing mutagenized DNA originating specifically from the region of interest to an untreated recipient cell. This can be achieved either by mutagenizing phage lysates prior to transduction or by transducing the region of interest to a new cell after mutagenizing the donor. Either of these approaches can be used if a selectable linked marker which is cotransducible with the target gene is available. Hong and Ames (71) mutagenized P22 in vitro with hydroxylamine and isolated temperature-sensitive mutations linked to auxotrophic markers in a recipient by selecting for transduction to prototrophy and screening the transductants for mutant phenotype. More recent applications, of which that described in reference 55 is an example, usually rely on selection for a linked, transposon-encoded drug resistance marker. The avalability of the Singer et al. (165; see also chapter 109) collection of strains with mapped Tn*10* elements greatly facilitates the use of this technique with the *E. coli*-P1 system.

Moving Genes. The Singer collection can also be used to facilitate the moving of alleles between strains. An appropriately located Tn*10* is introduced to the donor strain by transduction, and progeny are screened to find those in which it is now linked to the allele to be donated. A further lysate is then prepared, and transductants for the resistance marker are selected in the final recipient. It is necessary to ascertain at each stage that the transposon is still in its original position, since transposition can occur at the time of transduction. Berg et al. (12) and Kleckner et al. (92) have noted, respectively, that Tn*5* and Tn*10* can transpose to P1 with significant frequency during phage development in the donor strain. It also seems likely that transposition to new positions can occur after infection of the recipient. If the transduced strain is to be used as a donor in its turn, it is thus advisable to verify that the transposable element is still linked to the gene of interest.

The right- and left-hand portions of a transducing fragment can pair separately with noncontiguous DNA, allowing deletions to be transduced easily. It is also possible to construct new deletions or, more usually, replacements, with the aid of transduction. DNAs corresponding to the desired ends of the deletions are cloned, separated by a selective marker, on a plasmid or lysogenizing phage, and introduced to a donor strain. As described above, T4 will transduce plasmid DNA between strains of *E. coli;* P22 HT mutants could be used with *S. typhimurium*. Prophage DNA will be transduced by P1. Examples of these techniques can be found in references 84, 96, and 122. Transduction can also, of course, be used to move, from strain to strain, the rich variety of transposon and Mu-induced mutations now available. P1 transduction has also been used to transfer chromosomal markers to plasmids containing homologous regions (105). Since successful transfer requires RecA function, it has been supposed that transiently integrated plasmids are transduced and resolved in the recipient, with occasional exchange of alleles.

Gene Transfer between *E. coli* and *S. typhimurium*. Although F-plasmid-mediated conjugation between *E. coli* and *S. typhimurium* occurs efficiently (provided that rough strains are used as recipients and host-mediated restriction is avoided) (150), there are several barriers to intergeneric transduction. First, the favored generalized transducers, P1 and P22, do not infect both hosts. This problem can be overcome (see above) by using cell wall-mutant recipients to allow P1 adsorption to *S. typhimurium* (51, 57, 129) or by using a plasmid to introduce the genes necessary to synthesize P22 receptor into *E. coli* (126). Second, the evolutionary distance between the two species of bacteria means that sequence divergence strongly limits chromosomal recombination; most of the progeny recovered from crosses have undergone recombination at duplicated or highly conserved regions such as *rrn* loci (102). The discovery (143) that the barrier to recombinant recovery is mismatch repair of donor DNA during the recombinational process has provided

the key to performing interspecific crosses. *Salmonella* mismatch repair mutants will yield, in crosses with *E. coli*, recombinants for chromosomal markers at frequencies approaching that found in intraspecific crosses.

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LITERATURE CITED

1. **Arber, W.** 1960. Transduction of chromosomal genes and episomes in *Escherichia coli. Virology* **11:**273–288.
2. **Austin, S., F. Hart, A. Abeles, and N. Sternberg.** 1982. Genetic and physical map of a P1 miniplasmid. *J. Bacteriol.* **152:**63–71.
3. **Austin, S., M. Ziese, and N. Sternberg.** 1981. A novel role for site-specific recombination in maintenance of bacterial replicons. *Cell* **25:**729–736.
4. **Bächi, B., and W. Arber.** 1977. Physical mapping of *Bgl*II, *Bam*HI, *Eco*RI, *Hin*dIII and *Pst*I restriction fragments of bacteriophage P1 DNA. *Mol. Gen. Genet.* **153:**311–324.
5. **Backhaus, H.** 1985. DNA packaging initiation of *Salmonella* bacteriophage P22: determination of cut sites within the DNA sequence coding for gene *3. J. Virol.* **55:**458–465.
6. **Bade, E. G., M. M. Howe, and L. Rawluk.** 1978. Preferential generalized transduction by bacteriophage Mu. *Mol. Gen. Genet.* **160:**89–94.
7. **Balbinder, E.** 1962. The fine structure of the loci *tryC* and *tryD* of *Salmonella typhimurium.* I. Determination of the order of mutational sites by three-point transduction tests. *Genetics* **47:**469–482.
8. **Bender, R. A., and L. C. Sambucetti.** 1983. Recombination-induced suppression of cell division following P1-mediated generalized transduction in *Klebsiella aerogenes. Mol. Gen. Genet.* **189:**263–268.
9. **Bendig, M. M., and H. Drexler.** 1977. Transduction of bacteriophage Mu by bacteriophage T1. *J. Virol.* **22:**640–645.
10. **Benson, N. R., and B. S. Goldman.** 1992. Rapid mapping in *Salmonella typhimurium* with Mu*d*-P22 prophages. *J. Bacteriol.* **174:**1673–1681.

10a. **Benson, N. R., and J. Roth.** 1994. Suppressors of *recB* mutations in *Salmonella typhimurium*. *Genetics* **138:**11–28.

11. **Benzinger, R., and P. E. Hartman.** 1962. Effects of ultraviolet light on transducing phage P22. *Virology* **18:**614–626.
12. **Berg, C. M., C. A. Grullón, A. Wang, W. A. Whalen, and D. E. Berg.** 1983. Transductional instability of Tn*5*-induced mutations: generalized and specialized transduction of Tn*5* by bacteriophage P1. *Genetics* **105:**259–263.
13. **Bertani, G.** 1951. Studies in lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli. J. Bacteriol.* **62:**293–300.
14. **Black, L. W.** 1989. DNA packaging in dsDNA bacteriophages. *Annu. Rev. Microbiol.* **43:**267–292.
15. **Blume, A. J., and E. Balbinder.** 1966. The tryptophan operon of *Salmonella typhimurium.* Fine structure analysis by deletion mapping and abortive transduction. *Genetics* **53:**577–592.
16. **Boro, H., and J. E. Brenchley.** 1971. A new generalized transducing phage for *Salmonella typhimurium* LT2. *Virology* **45:**835–836.

17. **Botstein, D., and M. J. Matz.** 1970. A recombination function essential to the growth of bacteriophage P22. *J. Mol. Biol.* **54:**417–440.
18. **Botstein, D., C. H. Waddell, and J. King.** 1973. Mechanism of head assembly and DNA encapsulation in *Salmonella* phage P22. I. Genes, proteins, structures, and DNA maturation. *J. Mol. Biol.* **80:**669–695.
19. **Burns, R. O., J. Calvo, P. Margolin, and H. E. Umbarger.** 1966. Expression of the leucine operon. *J. Bacteriol.* **91:**1570–1576.
20. **Calvo, J. M., and H. E. Worden.** 1970. A multisite-mutation map of the leucine operon of *Salmonella typhimurium. Genetics* **64:**199–214.
21. **Caro, L., and C. M. Berg.** 1969. Chromosome replication in *Escherichia coli.* II. Origin of replication in $F^-$and F^+ strains. *J. Mol. Biol.* **45:**325–336.
22. **Casjens, S.** 1979. Molecular organization of the bacteriophage P22 coat protein shell. *J. Mol. Biol.* **131:**1–19.
23. **Casjens, S.** 1990. Bacteriophage P22 DNA packaging, p. 241–261. *In* K. W. Adolph (ed.), *Chromosomes: Eukaryotic, Prokaryotic, and Viral,* vol. III. CRC Press, Boca Raton, Fla.
24. **Casjens, S., and M. Hayden.** 1988. Analysis *in vivo* of the bacteriophage P22 headful nuclease. *J. Mol. Biol.* **199:**467–474.
25. **Casjens, S., and W. M. Huang.** 1982. Initiation of sequential packaging of bacteriophage P22 DNA. *J. Mol. Biol.* **157:**287–298.
26. **Casjens, S., W. M. Huang, M. Hayden, and R. Parr.** 1987. Initiation of bacteriophage P22 DNA packaging series. Analysis of a mutant that alters the DNA target specificity of the packaging apparatus. *J. Mol. Biol.* **194:**411–422.
27. **Casjens, S., L. Sampson, S. Randall, K. Eppler, H. Wu, J. B. Petri, and H. Schmieger.** 1992. Molecular genetic analysis of bacteriophage P22 gene *3* product, a protein involved in the initiation of headful DNA packaging. *J. Mol. Biol.* **227:**1086–1099.
28. **Casjens, S., E. Wyckoff, M. Hayden, L. Sampson, K. Eppler, S. Randall, E. T. Moreno, and P. Serwer.** 1992. Bacteriophage P22 portal protein is part of the gauge that regulates packing density of intravirion DNA. *J. Mol. Biol.* **224:**1055–1074.
29. **Chelala, C. A., and P. Margolin.** 1974. Effects of deletions on cotransduction linkage in *Salmonella typhimurium:* evidence that bacterial chromosome deletions affect the formation of transducing DNA fragments. *Mol. Gen. Genet.* **131:**97–112.
30. **Chelala, C. A., and P. Margolin.** 1976. Evidence that HT mutant strains of bacteriophage P22 retain an altered form of substrate specificity in the formation of transducing particles in *Salmonella typhimurium. Genet. Res.* **27:**315–322.
31. **Chesney, R. H., and J. R. Scott.** 1975. Superinfection immunity and prophage repression in phage P1. II. Mapping of the immunity-difference and ampicillin-resistance loci of phage P1 and ϕ*amp. Virology* **67:**375–384.
32. **Childs, J. D., and H. C. Birnboim.** 1984. Plasmids containing multiple copies of cloned DNA for use as DNA size markers for gel electrophoresis, constructed by bacteriophage T4 transduction. *Plasmid* **11:**82–91.
33. **Clark, A. J.** 1991. *rec* genes and homologous recombination proteins in *Escherichia coli. Biochimie* **73:**523–532.
34. **Clark, A. J., and K. B. Low.** 1988. Pathways and systems of homologous recombination in *Escherichia coli,* p. 155–215. *In* K. B. Low (ed.), *The Recombination of Genetic Material.* Academic Press, Inc., New York.
35. **Cohen, A., and A. J. Clark.** 1986. Synthesis of linear plasmid multimers in *Escherichia coli* K-12. *J. Bacteriol.* **167:**327–335.
36. **Cosloy, S. D.** 1982. Analysis of genetic recombination by the RecBC and RecF pathways of *Escherichia coli* K12, p. 261–273. *In* U. N. Streips, S. H. Goodgal, W. R. Guild, and G. A. Wilson (ed.),

Genetic Exchange. Marcel Dekker, Inc., New York.
37. **Demerec, M., and Z. Hartman.** 1956. Tryptophan mutants in *Salmonella typhimurium.* Genetic studies with bacteria. *Carnegie Inst. Washington Publ.* **612:**5–33.
38. **Demerec, M., and H. Ozeki.** 1959. Tests for allelism among auxotrophs of *Salmonella typhimurium. Genetics* **44:**269–278.
39. **Demerec, M., I. Blomstrand, and Z. E. Demerec.** 1955. Evidence of complex loci in *Salmonella. Proc. Natl. Acad. Sci. USA* **41:**359–364.
40. **Dixon, D. A., and S. C. Kowalczykowski.** 1993. The recombination hotspot χ is a regulatory sequence that acts by attenuating the nuclease activity of the *E. coli* RecBCD enzyme. *Cell* **73:**87–96.
41. **Dower, N. A., and F. W. Stahl.** 1981. χ activity during transduction-associated recombination. *Proc. Natl. Acad. Sci. USA* **78:**7033–7037.
42. **Drexler, H.** 1970. Transduction by bacteriophage T1. *Proc. Natl. Acad. Sci. USA* **66:**1083–1088.
43. **Drexler, H.** 1977. Specialized transduction of the biotin region of *Escherichia coli* by phage T1. *Mol. Gen. Genet.* **152:**59–63.
44. **Drexler, H.** 1984. Initiation by bacteriophage T1 of DNA packaging at a site between the *P* and *Q* genes of bacteriophage λ. *J. Virol.* **49:**754–759.
45. **Drexler, H.** 1988. Bacteriophage T1, p. 235—258. *In* R. Calendar (ed.), *The Bacteriophages,* vol. 1. Plenum Press, New York.
46. **Drexler, H., and K. J. Kylberg.** 1975. Effect of UV irradiation on transduction by coliphage T1. *J. Virol.* **16:**263–266.
47. **Earnshaw, W., S. Casjens, and S. C. Harrison.** 1976. Assembly of the head of bacteriophage P22: X-ray diffraction from heads, proheads and related structures. *J. Mol. Biol.* **104:**387–410.
48. **Ebel-Tsipis, J., D. Botstein, and M. S. Fox.** 1972. Generalized transduction by phage P22 in *Salmonella typhimurium.* I. Molecular origin of transducing DNA. *J. Mol. Biol.* **71:**433–448.
49. **Ebel-Tsipis, J., M. S. Fox, and D. Botstein.** 1972. Generalized transduction by bacteriophage P22 in *Salmonella typhimurium.* II. Mechanism of integration of transducing DNA. *J. Mol. Biol.* **71:**449–469.
50. **Enomoto, M.** 1967. Composition of chromosome fragments participating in phage P22-mediated transduction of *Salmonella typhimurium. Virology* **33:**474–482.
51. **Enomoto, M., and B. A. D. Stocker.** 1974. Transduction by phage P1*kc* in *Salmonella typhimurium. Virology* **60:**503–514.
52. **Faelen, M., A. Toussaint, and A. Resibois.** 1979. Mini-Muduction: a new mode of gene transfer mediated by mini-Mu. *Mol. Gen. Genet.* **176:**191–197.
53. **Franklin, N. C.** 1969. Mutation in *gal U* gene of *E. coli* blocks phage P1 infection. *Virology* **38:**189–191.
54. **Garen, A., and N. D. Zinder.** 1955. Radiological evidence for partial genetic homology between bacteriophage and host bacteria. *Virology* **1:**347–376.
55. **Gibbs, T. W., D. R. Gill, and G. P. C. Salmond.** 1992. Localized mutagenesis of the *fts YEX* operon: conditionally lethal missense substitutions in the FtsE cell division protein of *Escherichia coli* are similar to those found in the cystic fibrosis transmembrane conductance regulator protein (CFTR) of human patients. *Mol. Gen. Genet.* **234:**121–128.
56. **Gill, G. S., and L. A. MacHattie.** 1976. Limited permutations of the nucleotide sequence in bacteriophage T1 DNA. *J. Mol. Biol.* **104:**505–515.
57. **Goldberg, R. B., R. A. Bender, and S. L. Streicher.** 1974. Direct selection for P1-sensitive mutants of enteric bacteria. *J. Bacteriol.* **118:**810–814.
58. **Gross, J., and E. Englesberg.** 1959. Determination of the order of mutational sites governing L-arabinose utilization in *Escherichia coli* B/r by transduction with phage P1bt. *Virology* **9:**314–331.
59. **Hanks, M. C., and M. Masters.** 1987. Transductional analysis of chromosome replication time. *Mol. Gen. Genet.* **210:**288–293.
60. **Hanks, M. C., B. Newman, I. R. Oliver, and M. Masters.** 1988. Packaging of transducing DNA by

bacteriophage P1. *Mol. Gen. Genet.* **214:**523–532.
61. **Harriman, P. D.** 1972. A single-burst analysis of the production of P1 infectious and transducing particles. *Virology* **48:**595–600.
62. **Harshey, R. M.** 1988. Phage Mu, p. 193–234. *In* R. Calendar (ed.), *The Bacteriophages,* vol. 1. Plenum Press, New York.
63. **Hartman, P. E.** 1956. Linked loci in the control of consecutive steps in the primary pathway of histidine synthesis in *Salmonella typhimurium.* Genetic studies with bacteria. *Carnegie Inst. Washington Publ.* **612:**35–61.
64. **Hartman, P. E., Z. Hartman, and D. Šerman.** 1960. Complementation mapping by abortive transduction of histidine requiring *Salmonella* mutants. *J. Gen. Microbiol.* **22:**354–368.
65. **Hartman, P. E., Z. Hartman, R. C. Stahl, and B. N. Ames.** 1971. Classification and mapping of spontaneous and induced mutations in the histidine operon of *Salmonella. Adv. Genet.* **16:**1–34.
66. **Hartman, P. E., and A. W. Kozinski.** 1962. Effects of P^{32} decay on transduction by *Salmonella* phage P22. *Virology* **17:**233–244.
67. **Hartweig, E., C. Bazinet, and J. King.** 1986. DNA injection apparatus of phage P22. *Biophys. J.* **49:**24–26.
68. **Helling, R. B.** 1969. Recognition of altered deoxyribonucleic acid in recombination. *J. Bacteriol.* **100:**224–230.
69. **Helling, R. B.** 1973. Ultraviolet light-induced recombination. *Biochem. Biophys. Res. Commun.* **55:**752–757.
70. **Hoffman, B., and M. Levine.** 1975. Bacteriophage P22 virion protein which performs an essential early function. II. Characterization of the gene *16* function. *J. Virol.* **16:**1547–1559.
71. **Hong, J.-S., and B. N. Ames.** 1971. Localized mutagenesis of any specific small region of the bacterial chromosome. *Proc. Natl. Acad. Sci. USA* **68:**3158–3162.
72. **Howe, M. M.** 1973. Transduction by bacteriophage Mu-1. *Virology* **55:**103–117.
73. **Iida, S., and W. Arber.** 1977. Plaque forming specialized transducing phage P1: isolation of P1CmSmSu, a precursor of P1Cm. *Mol. Gen. Genet.* **153:**259–269.
74. **Iida, S., J. Meyer, and W. Arber.** 1978. The insertion element Is*1* is a natural constituent of coliphage P1 DNA. *Plasmid* **1:**357–365.
75. **Iida, S., J. Meyer, and W. Arber.** 1987. Mechanisms involved in the formation of plaque-forming derivatives from over-sized hybrid phages between bacteriophage P1 and the R plasmid NR1. *FEMS Microbiol. Lett.* **43:**117–120.
76. **Iida, S., M. B. Streiff, T. A. Bickle, and W. Arber.** 1987. Two DNA antirestriction systems of bacteriophage P1, *darA,* and *darB:* characterization of *darA*$^-$ phages. *Virology* **157:**156–166.
77. **Ikeda, H., M. Inuzuka, and J. Tomizawa.** 1970. P1-like plasmid in *Escherichia coli* 15. *J. Mol. Biol.* **50:**457–470.
78. **Ikeda, H., and J. Tomizawa.** 1965. Transducing fragments in generalized transduction by phage P1. I. Molecular origin of the fragments. *J. Mol. Biol.* **14:**85–109.
79. **Ikeda, H., and J. Tomizawa.** 1965. Transducing fragments in generalized transduction by phage P1. II. Association of DNA and protein in the fragments. *J. Mol. Biol.* **14:**110–119.
80. **Ikeda, H., and J. Tomizawa.** 1965. Transducing fragments in generalized transduction by phage P1. III. Studies with small phage particles. *J. Mol. Biol.* **14:**120–129.
81. **Ikeda, H., and J. Tomizawa.** 1968. Prophage P1, an extrachromosomal replication unit. *Cold Spring Harbor Symp. Quant. Biol.* **33:**791–798.
82. **Israel, J. V., T. F. Anderson, and M. Levine.** 1967. *In vitro* morphogenesis of phage P22 from heads and base-plate parts. *Proc. Natl. Acad. Sci. USA* **57:**284–291.
83. **Israel, V.** 1977. E proteins of bacteriophage P22. I. Identification and ejection from wild-type and defective particles. *J. Virol.* **23:**91–97.
84. **Izuhara, M., and R. Takata.** 1990. Site-directed insertion mutagenesis with cloned fragments in

Escherichia coli by P1 phage transduction. *Mol. Gen. Genet.* **220:**339–340.
85. **Jackson, E. N., D. A. Jackson, and R. J. Deans.** 1978. *Eco*RI analysis of bacteriophage P22 DNA packaging. *J. Mol. Biol.* **118:**365–388.
86. **Jackson, E. N., F. Laski, and C. Andres.** 1982. Bacteriophage P22 mutants that alter the specificity of DNA packaging. *J. Mol. Biol.* **154:**551–563.
87. **Jackson, E. N., H. I. Miller, and M. L. Adams.** 1978. *Eco*RI restriction endonuclease cleavage site map of bacteriophage P22. *J. Mol. Biol.* **118:**347–363.
88. **Johnston, H. M., and J. R. Roth.** 1981. Genetic analysis of the histidine operon control region of *Salmonella typhimurium. J. Mol. Biol.* **145:**713–734.
89. **Karlsson, M. B., M. Pirhonen, H. T. Saarilahti, and E. T. Palva.** 1991. Molecular cloning of *ompRS,* a regulatory locus controlling production of outer membrane proteins in *Erwinia carotovora* subsp. *carotovora. Mol. Gen. Genet.* **226:**353–360.
90. **Karu, A. E., and E. D. Belk.** 1982. Induction of *E. coli recA* protein via *recBC* and alternate pathways: quantitation by enzyme-linked immunosorbent assay (ELISA). *Mol. Gen. Genet.* **185:**275–282.
91. **Kemper, J.** 1974. Gene order and co-transduction in the *leu-ara-fol-pyrA* region of the *Salmonella typhimurium* linkage map. *J. Bacteriol.* **117:**94–99.
92. **Kleckner, N., D. F. Barker, D. G. Ross, and D. Botstein.** 1978. Properties of the translocatable tetracycline-resistance element Tn*10* in *Escherichia coli* and bacteriophage lambda. *Genetics* **90:**429–461.
93. **Kondo, E., D. K. Haapala, and S. Falkow.** 1970. The production of chloramphenicol acetyltransferase by bacteriophage P1CM. *Virology* **40:**431–440.
94. **Krajewska-Grynkiewicz, K., and T. Klopotowski.** 1979. Altered linkage values in phage P22-mediated transduction caused by distant deletions or insertions in donor chromosomes. *Mol. Gen. Genet.* **176:**87–93.
95. **Kufer, B., H. Backhaus, and H. Schmieger.** 1982. The packaging initiation site of phage P22. Analysis of packaging events by transduction. *Mol. Gen. Genet.* **187:**510–515.
96. **Kulakauskas, S., P. M. Wikström, and D. E. Berg.** 1991. Efficient introduction of cloned mutant alleles into the *Escherichia coli* chromosome. *J. Bacteriol.* **173:**2633–2638.
97. **Kuo, T.-T., and B. A. D. Stocker.** 1970. ES18, a general transducing phage for smooth and nonsmooth *Salmonella typhimurium. Virology* **42:**621–632.
98. **Kylberg, K. J., M. M. Bendig, and H. Drexler.** 1975. Characterization of transduction by bacteriophage T1: time of production and density of transducing particles. *J. Virol.* **16:**854–858.
99. **Labaw, L. W.** 1952. The origin of phosphorus in the T1, T5, T6 and T7 bacteriophage of *E. coli. J. Bacteriol.* **66:**429–436.
100. **Laski, F., and E. N. Jackson.** 1982. Maturation cleavage of bacteriophage P22 DNA in the absence of DNA packaging. *J. Mol. Biol.* **154:**565–579.
101. **Lederberg, J.** 1956. Linear inheritance in transductional clones. *Genetics* **41:**845–871.
102. **Lehner, A. F., and C. W. Hill.** 1985. Merodiploidy in *Escherichia coli–Salmonella typhimurium* crosses: the role of unequal recombination between ribosomal RNA genes. *Genetics* **110:**365–380.
103. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. *Virology* **1:**190–206.
104. **Liebeschuetz, J., and D. A. Ritchie.** 1986. Phage T1-mediated transduction of a plasmid containing the T1 *pac* site. *J. Mol. Biol.* **192:**681–692.
105. **Liljeström, P., M. Pirhonen, and E. T. Palva.** 1985. In vivo transfer of chromosomal mutations onto multicopy plasmids by transduction with bacteriophage P1. *Gene* **40:**241–246.
106. **Lindsey, D. F., C. Martínez, and J. R. Walker.** 1992. Physical map location of the *Escherichia coli* attachment site for the P22 prophage (*attP22*). *J. Bacteriol.* **174:**3834–3835.
107. **Lloyd, R. G., S. M. Picksley, and C. Prescott.** 1983. Inducible expression of a gene specific to the

RecF pathway for recombination in *Escherichia coli* K12. *Mol. Gen. Genet.* **190:**162–167.
108. **Lloyd, R. G., M. C. Porton, and C. Buckman.** 1988. Effect of *recF, recJ, recN, recO* and *ruv* mutations on ultraviolet survival and genetic recombination in a *recD* strain of *Escherichia coli* K12. *Mol. Gen. Genet.* **212:**317–324.
109. **Lloyd, R. G., and A. Thomas.** 1983. On the nature of the RecBC and RecF pathways of conjugal recombination in *Escherichia coli. Mol. Gen. Genet.* **190:**156–161.
110. **Luria, S. E., J. N. Adams, and R. C. Ting.** 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. *Virology* **12:**348–390.
111. **MacHattie, L. A., and G. S. Gill.** 1977. DNA maturation by the "headful" mode in bacteriophage T1. *J. Mol. Biol.* **110:**441–465.
112. **MacHattie, L. A., M. Rhoades, and C. A. Thomas, Jr.** 1972. Large repetition in the non-permuted nucleotide sequence of bacteriophage T1. *DNA* **72:**645–656.
113. **Margolin, P.** 1963. Genetic fine structure of the leucine operon in *Salmonella. Genetics* **48:**441–457.
114. **Margolin, P.** 1987. Generalized transduction, p. 1154–1168. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology.* American Society for Microbiology, Washington, D.C.
115. **Margolin, P., and R. H. Bauerle.** 1966. Determinants for regulation and initiation of expression of tryptophan genes. *Cold Spring Harbor Symp. Quant. Biol.* **31:**311–320.
116. **Masters, M.** 1970. Origin and direction of replication of the chromosome of *E. coli* B/r. *Proc. Natl. Acad. Sci. USA* **65:**601–608.
117. **Masters, M.** 1977. The frequency of P1 transduction of the genes of *Escherichia coli* as a function of chromosomal position: preferential transduction of the origin of replication. *Mol. Gen. Genet.* **155:**197–202.
118. **Masters, M.** 1985. Generalized transduction, p. 197–215. *In* J. Scaife, D. Leach, and A. Galizzi (ed.), *Genetics of Bacteria.* Academic Press, London.
119. **Masters, M., and P. Broda.** 1971. Evidence for the bidirectional replication of the *Escherichia coli* chromosome. *Nature* (London) *New Biol.* **232:**137–140.
120. **Masters, M., B. J. Newman, and C. M. Henry.** 1984. Reduction of marker discrimination in transductional recombination. *Mol. Gen. Genet.* **196:**85–90.
121. **McIntire, S. A.** 1974. Transduction with integration-defective mutants of *Salmonella typhimurium* bacteriophage KB1. *J. Bacteriol.* **117:**907–908.
121a. **McKane, M., and R. Milkman.** 1995. Transduction, restriction and recombination patterns in *Escherichia coli*. *Genetics* **139:**35–43.
122. **McLennan, N. F., A. S. Girshovich, N. M. Lissin, Y. Charters, and M. Masters.** 1993. The strongly conserved carboxyl-terminus glycine-methionine motif of the *Escherichia coli* GroEL chaperonin is dispensable. *Mol. Microbiol.* **7:**49–58.
123. **Miesel, L., and J. R. Roth.** 1994. *Salmonella recD* mutations increase recombination in a short sequence transduction assay. *J. Bacteriol.* **176:**4092–4103.
124. **Milkman, R., and M. M. Bridges.** 1993. Molecular evolution of the *Escherichia coli* chromosome. IV. Sequence comparisons. *Genetics* **133:**455–468.
125. **Mise, K., and W. Arber.** 1976. Plaque-forming transducing bacteriophage P1 derivatives and their behaviour in lysogenic conditions. *Virology* **69:**191–205.
126. **Neal, B. L., P. K. Brown, and P. R. Reeves.** 1993. Use of *Salmonella* phage P22 for transduction in *Escherichia coli. J. Bacteriol.* **175:**7115–7118.
127. **Newman, B. J., and M. Masters.** 1980. The variation in frequency with which markers are transduced by phage P1 is primarily a result of discrimination during recombination. *Mol. Gen. Genet.* **180:**585–589.
128. **Noguchi, T., and H. Takahashi.** 1993. Transactivation of a plasmid-borne bacteriophage T4 late

gene. *Mol. Gen. Genet.* **239:**393–399.
129. **Okada, M., and T. Watanabe.** 1968. Transduction with phage P1 in *Salmonella typhimurium. Nature* (London) **218:**185–187.
130. **Orbach, M. J., and E. N. Jackson.** 1982. Transfer of chimeric plasmids among *Salmonella typhimurium* strains by P22 transduction. *J. Bacteriol.* **149:**985–994.
131. **Ornellas, E. P., and B. A. D. Stocker.** 1974. Relation of lipopolysaccharide character to P1 sensitivity in *Salmonella typhimurium. Virology* **60:**491–502.
132. **Ozeki, H.** 1956. Abortive transduction in purine-requiring mutants of *Salmonella typhimurium.* Genetic studies with bacteria. *Carnegie Inst. Washington Publ.* **612:**97–106.
133. **Ozeki, H.** 1959. Chromosome fragments participating in transduction in *Salmonella typhimurium. Genetics* **44:**457–470.
134. **Ozeki, H., and H. Ikeda.** 1968. Transduction mechanisms. *Annu. Rev. Genet.* **2:**245–278.
135. **Pato, M. L.** 1989. Bacteriophage Mu, p. 23–52. *In* D. E. Berg and M. M. Howe (ed.), *Mobile DNA.* American Society for Microbiology, Washington, D.C.
136. **Pearce, U., and B. A. D. Stocker.** 1965. Variation in composition of chromosome fragments transduced by phage P22. *Virology* **27:**290–296.
137. **Plakidou, S., K. G. Moffat, G. P. C. Salmond, and G. MacKinnon.** 1984. Convenient transduction of *recA* with bacteriophage T4GT7. *J. Bacteriol.* **159:**1072–1073.
138. **Poteete, A. R.** 1988. Bacteriophage P22, p. 647–682. *In* R. Calendar (ed.), *The Bacteriophages,* vol. 2. Plenum Press, New York.
139. **Poteete, A. R., A. C. Fenton, and K. C. Murphy.** 1988. Modulation of *Escherichia coli* RecBCD activity by the bacteriophage λ Gam and P22 Abc functions. *J. Bacteriol.* **170:**2012–2021.
140. **Poteete, A. R., and J. King.** 1977. Functions of two new genes in *Salmonella* phage P22 assembly. *Virology* **76:**725–739.
141. **Raj, A. S., A. Y. Raj, and H. Schmieger.** 1974. Phage genes involved in the formation of generalized transducing particles in *Salmonella*-phage P22. *Mol. Gen. Genet.* **135:**175–184.
142. **Ramsey, N., and D. A. Ritchie.** 1980. A physical map of the permuted genome of bacteriophage T1. *Mol. Gen. Genet.* **179:**669–675.
143. **Rayssiguier, C., D. S. Thaler, and M. Radman.** 1989. The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature* (London) **342:**396–401.
144. **Ritchie, D. A., and D. H. Joicey.** 1978. Formation of concatemeric DNA as an intermediate in the replication of bacteriophage T1 DNA molecules. *J. Gen. Virol.* **41:**609–622.
145. **Roberts, M. D., and H. Drexler.** 1981. Isolation and genetic characterization of T1-transducing mutants with increased transduction frequency. *Virology* **112:**662–669.
146. **Roberts, M. D., and H. Drexler.** 1981. T1 mutants with increased transduction frequency are defective in host chromosome degradation. *Virology* **112:**670–677.
147. **Rosner, J. L.** 1972. Formation, induction and curing of bacteriophage P1 lysogens. *Virology* **48:**679–689.
148. **Roth, J. R., and P. E. Hartman.** 1965. Heterogeneity in P22 transducing particles. *Virology* **27:**297–307.
149. **Sanderson, K. E., and J. A. Hurley.** 1987. Linkage map of *Salmonella typhimurium,* p. 877–918. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology.* American Society for Microbiology, Washington, D.C.
150. **Sanderson, K. E., and P. R. MacLachlan.** 1987. F-mediated conjugation, F^+ strains, and Hfr strains of *Salmonella typhimurium* and *Salmonella abony,* p. 1138–1144. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology.* American Society for Microbiology, Washington, D.C.

151. **Sanderson, K. E., and J. R. Roth.** 1983. Linkage map of *Salmonella typhimurium,* edition VI. *Microbiol. Rev.* **47:**410–453.
152. **Sandri, R. M., and H. Berger.** 1980. Bacteriophage P1-mediated generalized transduction in *Escherichia coli:* fate of transduced DNA in Rec^+ and $RecA^-$ recipients. *Virology* **106:**14–29.
153. **Sandri, R. M., and H. Berger.** 1980. Bacteriophage P1-mediated generalized transduction in *Escherichia coli:* structure of abortively transduced DNA. *Virology* **106:**30–40.
154. **Schmidt, C., and H. Schmieger.** 1984. Selective transduction of recombinant plasmids with cloned *pac* sites by *Salmonella* phage P22. *Mol. Gen. Genet.* **196:**123–128.
155. **Schmieger, H.** 1968. Die molekulare Struktur transduzierender Partikel beim *Salmonella*-Phagen *P22.* I. Dichtegradienten-Untersuchungen an intakten Phagen. *Mol. Gen. Genet.* **102:**336–347.
156. **Schmieger, H.** 1970. The molecular structure of the transducing particles of *Salmonella* phage *P22.* II. Density gradient analysis of DNA. *Mol. Gen. Genet.* **109:**323–337.
157. **Schmieger, H.** 1971. The fate of the bacterial chromosome in *P22*-infected cells of *Salmonella typhimurium. Mol. Gen. Genet.* **110:**238–244.
158. **Schmieger, H.** 1972. Phage *P22*-mutants with increased or decreased transduction abilities. *Mol. Gen. Genet.* **119:**75–88.
159. **Schmieger, H.** 1982. Packaging signals for phage P22 on the chromosome of *Salmonella typhimurium. Mol. Gen. Genet.* **187:**516–518.
160. **Schmieger, H., and H. Backhaus.** 1976. Altered cotransduction frequencies exhibited by HT-mutants of *Salmonella*-phage P22. *Mol. Gen. Genet.* **143:**307–309.
161. **Schmieger, H., and U. Buch.** 1975. Appearance of transducing particles and the fate of host DNA after infection of *Salmonella typhimurium* with P22-mutants with increased transducing ability (HT-mutants). *Mol. Gen. Genet.* **140:**111–122.
162. **Schroeder, W., E. G. Bade, and H. Delius.** 1974. Participation of *Escherichia coli* DNA in the replication of temperate bacteriophage Mu1. *Virology* **60:**534–542.
163. **Scott, J. R.** 1980. Immunity and repression in bacteriophages P1 and P7. *Curr. Top. Microbiol. Immunol.* **90:**49–65.
164. **Sheppard, D. E.** 1962. Density gradient centrifugation of bacteriophage P22. *Virology* **17:**212–214.
165. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli. Microbiol. Rev.* **53:**1–24.
166. **Skorupski, K., J. C. Pierce, B. Sauer, and N. Sternberg.** 1992. Bacteriophage P1 genes involved in the recognition and cleavage of the phage packaging site (*pac*). *J. Mol. Biol.* **223:**977–989.
167. **Smith, G. R.** 1989. Homologous recombination in *E. coli:* multiple pathways for multiple reasons. *Cell* **58:**807–809.
168. **Smith, G. R.** 1991. Conjugational recombination in *E. coli:* myths and mechanisms. *Cell* **64:**19–27.
169. **Smith, H. W.** 1972. Ampicillin resistance in *Escherichia coli* by phage infection. *Nature* (London) *New Biol.* **238:**205–206.
170. **Starlinger, P.** 1959. Zur Herkunft des in der Transduktion durch den Phagen P22 übertragenen Materials. *Z. Naturforsch.* **146:**523–528.
171. **Sternberg, N.** 1986. The production of generalized transducing phage by bacteriophage lambda. *Gene* **50:**69–85.
172. **Sternberg, N.** 1990. Bacteriophage P1 DNA packaging, p. 225–240. *In* K. W. Adolph (ed.), *Chromosomes: Eukaryotic, Prokaryotic, and Viral,* vol. III. CRC Press, Boca Raton, Fla.
173. **Sternberg, N., and J. Coulby.** 1987. Recognition and cleavage of the bacteriophage P1 packaging site (*pac*). I. Differential processing of the cleaved ends *in vivo. J. Mol. Biol.* **194:**453–468.
174. **Sternberg, N., and J. Coulby.** 1987. Recognition and cleavage of the bacteriophage P1 packaging site (*pac*). II. Functional limits of *pac* and location of *pac* cleavage termini. *J. Mol. Biol.* **194:**469–479.

175. **Sternberg, N., and J. Coulby.** 1990. Cleavage of the bacteriophage P1 packaging site (*pac*) is regulated by adenine methylation. *Proc. Natl. Acad. Sci. USA* **87:**8070–8074.
176. **Sternberg, N., D. Hamilton, S. Austin, M. Yarmolinsky, and R. Hoess.** 1980. Site-specific recombination and its role in the life cycle of bacteriophage P1. *Cold Spring Harbor Symp. Quant. Biol.* **45:**297–309.
177. **Sternberg, N., and R. Weisberg.** 1975. Packaging of prophage and host DNA by coliphage λ. *Nature* (London) **256:**97–103.
178. **Sternberg, N. L., and R. Maurer.** 1991. Bacteriophage-mediated generalized transduction in *Escherichia coli* and *Salmonella typhimurium. Methods Enzymol.* **204:**18–43.
179. **Stocker, B. A. D.** 1956. Abortive transduction of motility in *Salmonella:* a nonreplicated gene transmitted through many generations to a single descendant. *J. Gen. Microbiol.* **15:**575–598.
180. **Stocker, B. A. D., N. D. Zinder, and J. Lederberg.** 1953. Transduction of flagellar characters in *Salmonella. J. Gen. Microbiol.* **9:**410–433.
181. **Strauss, H., and J. King.** 1984. Steps in stabilization of newly packaged DNA during phage-P22 morphogenesis. *J. Mol. Biol.* **172:**523–543.
182. **Streisinger, G., R. S. Edgar, and G. H. Denhardt.** 1964. Chromosome structure in phage T4. I. Circularity of the linkage map. *Proc. Natl. Acad. Sci. USA* **51:**775–779.
183. **Streisinger, G., J. Emrich, and M. M. Stahl.** 1967. Chromosome structure in phage T4. III. Terminal redundancy and length determination. *Proc. Natl. Acad. Sci. USA* **57:**292–295.
184. **Susskind, M. M., and D. Botstein.** 1978. Molecular genetics of bacteriophage P22. *Microbiol. Rev.* **42:**385–413.
185. **Symonds, N., A. Toussaint, P. van de Putte, and M. M. Howe (ed.).** 1987. *Phage Mu.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
186. **Takahashi, H., and H. Saito.** 1982. High-frequency transduction of pBR322 by cytosine-substituted T4 bacteriophage: evidence for encapsulation and transfer of head-to-tail plasmid concatemers. *Plasmid* **8:**29–35.
187. **Takahashi, H., and H. Saito.** 1982. Mechanism of pBR322 transduction mediated by cytosine-substituting T4 bacteriophage. *Mol. Gen. Genet.* **186:**497–500.
188. **Takebe, H., and P. E. Hartman.** 1962. Effect of X-rays on transduction by *Salmonella* phage P22. *Virology* **17:** 295–300.
189. **Teifel, J., and H. Schmieger.** 1979. The origin of the DNA in transducing particles of bacteriophage Mu. *Mol. Gen. Genet.* **176:**293–295.
190. **Teifel, J., and H. Schmieger.** 1979. Phage Mu mutants with increased transduction abilities. *Intervirology* **11:**314–316.
191. **Teifel, J., and H. Schmieger.** 1981. The influence of host DNA replication on the formation of infectious and transducing Mu-particles. *Mol. Gen. Genet.* **184:**308–311.
192. **Tye, B.-K., R. K. Chan, and D. Botstein.** 1974. Packaging of an oversize transducing genome by *Salmonella* phage P22. *J. Mol. Biol.* **85:**485–500.
193. **Tye, B.-K., J. A. Huberman, and D. Botstein.** 1974. Non-random circular permutation of phage P22 DNA. *J. Mol. Biol.* **85:**501–532.
194. **Vogel, W., and H. Schmieger.** 1986. Selection of bacterial *pac* sites recognized by *Salmonella* phage P22. *Mol. Gen. Genet.* **205:**563–567.
195. **Walker, D. H., Jr., and T. F. Anderson.** 1970. Morphological variants of coliphage P1. *J. Virol.* **5:**765–782.
196. **Walker, J. T., S. Iida, and D. H. Walker.** 1979. Permutation of the DNA in small-headed virions of coliphage P1. *Mol. Gen. Genet.* **167:**341–344.
197. **Walker, J. T., and D. H. Walker, Jr.** 1981. Structural proteins of coliphage P1. *Prog. Clin. Biol. Res.* **64:**69–77.
198. **Walker, J. T., and D. H. Walker, Jr.** 1983. Coliphage P1 morphogenesis: analysis of mutants by

electron microscopy. *J. Virol.* **45:**1118–1139.
199. **Wall, J. D., and P. D. Harriman.** 1974. Phage P1 mutants with altered transducing abilities for *Escherichia coli. Virology* **59:**532–544.
200. **Wandersman, C., and M. Yarmolinsky.** 1977. Bipartite control of immunity conferred by the related heteroimmune plasmid prophages, P1 and P7 (formerly ϕAmp). *Virology* **77:**386–400.
201. **Weaver, S., and M. Levine.** 1977. The timing of *erf*-mediated recombination in replication, lysogenization and the formation of recombinant progeny by *Salmonella* phage P22. *Virology* **76:**19–28.
202. **Weaver, S., and M. Levine.** 1978. Replication *in situ* and DNA encapsulation following induction of an excision-defective lysogen of *Salmonella* bacteriophage P22. *J. Mol. Biol.* **118:**389–411.
203. **West, S. C.** 1992. Enzymes and molecular mechanisms of genetic recombination. *Annu. Rev. Biochem.* **61:**603–640.
204. **Wilson, G. G., K. K. Y. Young, G. J. Edlin, and W. Konigsberg.** 1979. High frequency generalised transduction by bacteriophage T4. *Nature* (London) **280:**80–82.
205. **Wood, W. B., and H. R. Revel.** 1976. The genome of bacteriophage T4. *Bacteriol. Rev.* **40:**847–868.
206. **Wu, T. T.** 1966. A model for three-point analysis of random general transduction. *Genetics* **54:**405–410.
207. **Yamagami, H., and N. Yamamoto.** 1970. Contribution of the bacterial recombination function to replication of bacteriophage P22. *J. Mol. Biol.* **53:**281–285.
208. **Yamamoto, N., and T. F. Anderson.** 1961. Genomic masking and recombination between serologically unrelated phages P22 and P221. *Virology* **14:**430–439.
209. **Yamamoto, Y.** 1982. Phage P1 mutant with decreased abortive transduction. *Virology* **118:**329–344.
210. **Yanofsky, C., and E. S. Lennox.** 1959. Transduction and recombination study of linkage relationships among genes controlling tryptophan synthesis in *Escherichia coli. Virology* **8:**425–447.
211. **Yarmolinsky, M.** 1987. Bacteriophage P1, p. 38–47. *In* S. J. O'Brien (ed.), *Genetic Maps, 1987,* vol. 4. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
212. **Yarmolinsky, M. B., and N. Sternberg.** 1988. Bacteriophage P1, p. 291–438. *In* R. Calendar (ed.), *The Bacteriophages,* vol. 1. Plenum Press, New York.
213. **Youderian, P., P. Sugiono, K. L. Brewer, N. P. Higgins, and T. Elliott.** 1988. Packaging specific segments of the *Salmonella* chromosome with locked-in Mu*d*-P22 prophages. *Genetics* **118:**581–592.
214. **Young, K. K. Y., and G. Edlin.** 1983. Physical and genetical analysis of bacteriophage T4 generalized transduction. *Mol. Gen. Genet.* **192:**241–246.
215. **Young, K. K. Y., G. J. Edlin, and G. G. Wilson.** 1982. Genetic analysis of bacteriophage T4 transducing bacteriophages. *J. Virol.* **41:**345–347.
216. **Yun, T., and D. Vapnek.** 1977. Electron microscopic analysis of bacteriophages P1, P1Cm and P7. Determination of genome sizes, sequence homology and location of antibiotic-resistance determinants. *Virology* **77:**376–385.
217. **Zinder, N. D.** 1992. Forty years ago: the discovery of bacterial transduction. *Genetics* **132:**291–294.
218. **Zinder, N. D., and J. Lederberg.** 1952. Genetic exchange in *Salmonella. J. Bacteriol.* **64:**679–699.