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Genetic Analysis of Bacteriophage T4 Transducing Bacteriophages

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Mutations in the genes for nuclear disruption (*ndd*), endonuclease IV (*denB*), and the D_1 region of the T4 genome are essential for converting bacteriophage T4 into a generalized transducing phage. These mutations gave rise to a very low frequency of transduction, about 10^{-8} per infected bacterium. The addition of an *r*II mutation raised the transduction frequency about 20-fold. An additional 100-fold increase in the transduction frequency was observed with mutations in genes 42, 56, and *alc*. High-frequency generalized transduction by T4 results from the cumulative effect of these mutations.

A previous report showed that a particular mutant of bacteriophage T4 (GT7) possessing a number of genetic alterations is capable of high-frequency generalized transduction in *Escherichia coli* (10). The original mutant contains amber mutations in genes 42 (deoxycytidylate hydroxymethylase) and 56 (deoxycytidine triphosphatase) and the deletion NB5060, which deletes DNA extending from the *r*IIB gene through the *ac* gene for acriflavine uptake and resistance (2, 3, 11). The phage also has an *alc* mutation (*a*llows *l*ate transcription on *c*ytosine-containing DNA) that permits growth of the phage in *E. coli* strains that are incapable of supressing amber mutations (9). For a determination of which of the genetic modifications is required to convert the virulent T4 phage into a generalized transducing phage in *E. coli*, various T4 mutants were constructed and tested for their capacity to perform generalized transduction.

In this report we show that several mutations in one region of the T4 genome are required to produce phages capable of a very low, but measurable, level of transduction. The T4 genes involved are *ndd*, which is involved in bacterial nuclear disruption (8); *denB*, which codes for endonuclease IV (7); and the D_1 region, which may code for one or more proteins (1, 4). Mutations in either the *r*IIA or the *r*IIB cistron increased the frequency of transduction about 20-fold. Mutations in genes 42, 56, and *alc* increased the frequency of transduction another 100-fold.

Table 1 gives the transduction frequencies for T4 mutants in which various amounts of DNA in the *r*II-*ac* region of the T4 genome have been deleted. The *r*II deletion mutant NB5060, originally isolated by Benzer (2), transduced at a very low frequency, about 1/100 that of GT7 (Table 2). NB3157 contains an *r*IIB deletion that does not extend as far to the right as does NB5060. From the data of Bautz and Bautz (1) and Depew et al. (4), the right-hand endpoint of NB3157 was estimated to be in *ndd*. This deletion mutant transduced at about the same frequency as did NB5060, which suggests that mutations in the region spanning *r*IIB and *ndd* are essential for transduction by T4.

For further delineation of the mutation(s) in this region that is essential for transduction, recombinant T4 phages containing deletions of various sizes were constructed. T4 deletion mutants SaΔ4, SaΔ5, and 1272 by themselves did not transduce. However, the recombinants SaΔ5-1272 and SaΔ4-1272 transduced at about the same frequency as the long deletion mutants NB5060 and NB3157. SaΔ3 did not transduce by itself or in conjunction with deletion 1272, which suggests that the mutation in *ndd* and the mutation(s) in the *r*IIB-*denB* region are required to convert T4 into a transducing phage. This conclusion was reinforced by the finding that deletion mutants 1241 and SaΔ5-1241 did not transduce at a detectable level.

SaΔ9, a non-*r*II deletion mutant, extends from the D_1 region to *ac* (4). This mutant was found to carry out transduction at about 1/1,000 the frequency of GT7. This result, together with the findings indicated above, suggests that T4 gene *ndd* plus gene *denB*, and possibly genes in the D_1 region, need to be inactivated to permit generalized transduction by T4 phage at a low, but detectable, level.

Deletions residing solely within the *r*II region of T4 did not give arginine transductants at a detectable frequency (Table 1). T4 mutants were constructed by genetic crosses that coupled the *r*IIA or *r*IIB mutation with deletion SaΔ9. Muta-

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TABLE 1. Frequency of arginine transductants obtained with T4 deletion mutants in the *r*II-ac region

T4 deletion	*r*IIA	*r*IIB	D_1	*denB*	*pla262*	*ndd*	*stp*	*ac*	Transduction frequency (Arg^+)[a]
NB5060									1.5×10^{-7} (2.9×10^{-8})
NB3157									1.7×10^{-7} (2.6×10^{-8})
1272									$<10^{-9}$
SaΔ4									$<10^{-9}$
SaΔ5									$<10^{-9}$
SaΔ4-1272									1.1×10^{-7} (2.1×10^{-8})
SaΔ5-1272									1.2×10^{-7} (2.2×10^{-8})
SaΔ3									$<10^{-9}$
SaΔ3-1272									$<10^{-9}$
1241									$<10^{-9}$
SaΔ5-1241									$<10^{-9}$
SaΔ9									1.1×10^{-8} (1.8×10^{-9})
184									$<10^{-9}$
196									$<10^{-9}$
P53-SaΔ9		×							9.5×10^{-8} (2.4×10^{-8})
184-SAΔ9									2.2×10^{-7} (7.1×10^{-8})
196-SAΔ9									1.9×10^{-7}
184-5060									1.9×10^{-7} (3.9×10^{-8})

[a] Transduction frequencies are the average of at least two independent experiments (with the exception of strain 196-SaΔ9). The numbers in parentheses are the standard errors of the means. Transduction frequencies of $<10^{-9}$ mean that, of 10^{-9} infected cells plated, no transductant colonies were found. All phage stocks and phage crosses were prepared in *E. coli* QD *pro sup-3*. *E. coli* JC411 was the recipient bacterium in all transduction experiments. T4*r*II mutants were detected by their inability to grow on a QDλ lysogen. Acriflavine-resistant phages were selected by their ability to form plaques in the presence of 2 μg of acriflavine per ml.

tions in either the *r*IIA or the *r*IIB cistron raised the frequency of arginine transduction approximately 10-fold, provided that deletion SaΔ9 also is contained in the phage genome (Table 1). The mechanism whereby the loss of the *r*II function was able to increase the frequency of transduction is not understood.

Mutations that lie outside the *r*II-*ndd* region of the T4 genome also contributed to efficient transduction, since the frequencies shown in Table 1 were still 100 times lower than the transduction frequencies obtained with GT7. Table 2 gives the arginine transduction frequency obtained with GT7 and those for other genetically relevant mutant strains of T4. Individually, neither gene 42 nor gene 56 T4 mutants transduced, nor did they raise the frequency of transduction of deletion NB5060, which, by itself, was about 10^{-7}. However, the loss of gene 42 function must be required for high-frequency transduction, as shown by the 100-fold difference in transduction frequencies observed in experiments with phages GT4 and GT7. A mutation in the *alc* gene also increased the frequency of arginine transductants, since the genetic difference between T4 mutant E51-C87-NB5060 and GT7 was the loss of the *alc* function (Table 2). A note of caution should be added. Alc^- mutants are known to have a tendency to accumulate secondary mutations, such as deletions and insertions (E. Kutter, personal communication). Therefore, one might argue that the additional 100-fold increase in transduction frequency attributed to genes 42 and *alc* may, in fact, have been the result of secondary mutations. The fact that other independently isolated Alc^-

TABLE 2. Transduction frequencies with other T4 mutant strains

T4 phage strain	Genotype	Transduction frequency (Arg[a])
E51	*amg*56	$<10^{-9}$
C87	*amg*42	$<10^{-9}$
E51-NB5060	*am*56 Δ(*r*IIB-*ac*)	9.1×10^{-8} (5.3×10^{-9})
E51-C87-NB5060	*am*56 *am*42 Δ(*r*IIB-*ac*)	2.3×10^{-7} (7.7×10^{-8})
GT4	*am*56 Δ(*r*IIB-*ac*) *alc*	1.6×10^{-7} (2.4×10^{-8})
GT7	*am*56 *am*42 Δ(*r*IIB-*ac*) *alc*	1.3×10^{-5} (1.1×10^{-6})

[a] See Table 1, footnote *a*.

mutants showed the same 100-fold difference in transduction frequency (data not shown) makes this highly unlikely. If secondary mutations were involved, the different Alc$^-$ mutants would have to possess secondary mutations affecting similar functions, and this is unlikely.

The conversion of a virulent T4 phage into a phage capable of generalized transduction is accomplished by a number of genetic alterations in the T4 genome. The primary mutations essential for T4 transduction reside in T4 genes *ndd* and *denB* and an as yet unspecified gene(s) in the D_1 region. The transduction frequencies of the various deletion mutants listed in Tables 1 and 2 support the conclusion that the alteration of several genes in T4 is required to generate T4 transducing phage particles. The low frequency of transduction resulting from mutations in the D_1-*ndd* region was increased by mutations in either the *r*IIA or the *r*IIB cistron and genes 42 and *alc*.

For transduction by T4 phage, it seems to be necessary to maintain the integrity of the bacterial DNA, which is accomplished by mutations in T4 genes *ndd* and *denB*. Since no functions have been ascribed to genes in the D_1 region, the possible role of this region of the T4 genome in transduction remains obscure. It is not known whether transducing phage particles contain only bacterial DNA or a combination of bacterial and phage DNAs, and experiments are in progress to determine the nature of the DNA in transducing phage particles.

It is known that infection of *E. coli* by T4 phages which are incapable of reproducing in the bacteria due to genetic defects still results in the death of the infected cell (6). T4 phage ghosts are also able to effectively lyse bacteria to which they attach, even though they contain little or no phage DNA (5). Clearly, GT7 and other transducing T4 phages do not lyse the bacteria that they infect. T4 genes in the *r*II-*ac* region are involved in functions affecting the bacterial membrane (11). T4 transducing phages are being purified. Analysis of these purified phages should indicate (1) the nature of the DNA that they contain and (2) their ability to lyse bacteria that they infect.

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LITERATURE CITED

1. **Bautz, F. A., and E. K. F. Bautz.** 1967. Mapping of deletions in a non-essential region of the phage T4 genome. J. Mol. Biol. **28**:345–355.
2. **Benzer, S.** 1955. Fine structure of a genetic region in bacteriophage. Proc. Natl. Acad. Sci. U.S.A. **41**:344–355.
3. **Benzer, S.** 1961. On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. U.S.A. **47**:403–415.
4. **Depew, R. E., T. J. Snopek, and N. R. Cozzarelli.** 1975. Characterization of a new class of deletions of the D region of the bacteriophage T4 genome. Virology **64**:144–152.
5. **Duckworth, D. H.** 1970. Biological activity of bacteriophage ghosts and "take-over" of host functions by bacteriophage. Bacteriol. Rev. **34**:344–363.
6. **Koerner, J. F., and D. P. Snustad.** 1979. Shutoff of host macromolecular synthesis after T-even bacteriophage infection. Microbiol. Rev. **43**:199–223.
7. **Kutter, E., A. Beug, R. Sluss, L. Jensen, and D. Bradley.** 1975. The production of undegraded cytosine-containing DNA by bacteriophage T4 in the absence of dCTPase and endonucleases II and IV, and its effects on T4 directed protein synthesis. J. Mol. Biol. **99**:591–608.
8. **Snustad, D. P., C. J. H. Bursch, K. A. Parson, and S. H. Hefeneider.** 1976. Mutants of bacteriophage T4 deficient in the ability to induce nuclear disruption: shutoff of host DNA and protein synthesis, gene dosage experiments, identification of a restrictive host, and possible biological significance. J. Virol. **18**:268–288.
9. **Snyder, L., L. Gold, and E. M. Kutter.** 1976. A gene of bacteriophage T4 whose product prevents true late transcription on cytosine-containing DNA. Proc. Natl. Acad. Sci. U.S.A. **73**:3098–3102.
10. **Wilson, G. G., K. K. Y. Young, and G. J. Edlin.** 1979. High-frequency generalized transduction by bacteriophage T4. Nature (London) **280**:80–82.
11. **Wood, W. B., and H. R. Revel.** 1976. The genome of bacteriophage T4. Bacteriol. Rev. **40**:847–868.