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Phage *P22*-Mutants with Increased or Decreased Transduction Abilities

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Summary. The properties of mutants of the *Salmonella* phage *P22* are described which show decreased or increased frequencies for generalized transduction. It is shown that not only the markers used for detection of the mutants are affected by the mutation but also other markers tested. Evidence is presented that the altered T/P-ratios reflect changes in the actual numbers of transducing particles and that other factors like integration in the recipient cells cannot account for these alterations. Quantitative estimations of the amount of bacterial DNA converted to transducing fragments are shown for several phage mutants. Difficulties in the isolation procedure are discussed.

Introduction

A method has been described which allows the detection of phage mutants with increased (HT-mutants) or decreased (NT-mutants) transduction frequencies compared with those of wild type phages (Schmieger, 1971b). Using this method we isolated a number of independent mutants of both types. Here some of their general properties will be described.

Material and Methods

Bacteria. Donor strain in transduction experiments was wild type *Salmonella typhimurium LT2.* This strain and the recipients *His HB22 (P22), Try 8 (P22)* as well as the thymine-requiring mutant *T4/2* have already been described (Schmieger, 1970, 1971a). In addition we used strains *Cys E8 (P22), SU 453 (P22)* with the markers *met-A22 try-2 his-1009 xyl* S^R and *Thr-47 Leu-39 Ara-9 (P22)* which were obtained from Dr. K. E. Sanderson. From the latter strain *Leu-39 Ara-9 (P22)* was made by transduction to threonine-independence. *Arg (P22)* is a N-methyl-N′-nitro-N-nitrosoguanidine-induced mutant of *LT2* without specification. All auxotrophic mutants were used as *P22*-lysogens; so it was possible to transduce them with c^+- and c_2-phages.

Phages. P22H5, a c_2-clear plaque mutant of phage *P22,* was normally used in these experiments. Despite the c_2-mutation this strain is called in this paper "wild type" with reference to its transduction ability. NT- and HT-phage mutants with isolation numbers smaller than 100 were isolated from *P22H5,* those with numbers larger than 100 were isolated from $P22c^+$ by the method described (Schmieger, 1971b).

Media and Solutions. NB, NB-agar, M9, M9-agar and TCG have been described earlier (Schmieger, 1968, 1970). Amino acids were added to M9 and M9-agar at a concentration of 20 µg/ml wherever necessary. TCG/20 and TCG/200 means TCG + 20 µg and 200 µg thymidine/ml resp.

Buffered saline contained 0.1 M NaCl and 0.01 M Tris pH 7.4. Sodiumdodecylsulfate (SDS) was kept in a 10% solution.

Transduction. Transduction experiments were carried out by mixing NB-grown overnight cultures of the lysogenic recipient strains ($\sim 2 \times 10^9$ cells/ml) with the phage lysate at a moi ≤ 10 if not indicated otherwise. Adsorption was allowed for 10 min at 37° C. After appropriate dilution in NB aliquots were plated onto selective M9-agar plates. The plates were incubated for two days at 37° C.

Differential Labelling of DNA of Transducing and Infective Phages. For labelling NT-mutants an overnight culture of *T4/2* in TCG/20 was diluted 1/100 in TCG/20 and grown with aeration at 37° C to a concentration of 2×10^8 cells/ml. Then cells were centrifuged, washed and resuspended in 1/10 vol TCG without thymidine. This concentrated suspension was diluted 1/100 into TCG + 1 µg cold thymidine/ml + 10 µC ^{3}H-thymidine/ml + 250 µg deoxyadenosine/ml and again aerated at 37° C until it reached a concentration of 2×10^8 cells/ml. Then cells were centrifuged, resuspended in TCG/200 and aerated for 30 min to clean the pool from radioactive thymidine. At this time a sample of 0.3 ml was taken to estimate the 100% values in Tables 6 and 7. This sample was incubated with 1 mg pronase/ml and 1% SDS for 5 hours at 37° C. 0.1 ml aliquots were precipitated and prepared for counting in a liquid scintillation spectrometer as described below. Then 10 ml-aliquots of the prelabelled cells were added to the different phages and KCN solution. The phages were present at a multiplicity of 10 per cell. KCN was added to give a final concentration 2×10^{-3} M. Adsorption was allowed for 3 min at 37° C without aeration. Then cells were centrifuged, resuspended in 10 ml TCG/200 + 1 µC ^{32}P/ml and aerated for 1 hour. Chloroform was added and aeration continued until the cultures were cleared. The lysates were centrifuged and assayed for plaque-forming units. For purification, lysates were incubated with 100 µg/ml of DNase and 100 µg/ml of RNase for 1 hour in 37° C and then centrifuged on a stepwise CsCl-gradient ($\varrho = 1.6$ and 1.4 g $\times$ cm^{-3}) for 1 hour at 90000 $\times$ g. The zones between the two layers of CsCl which contained the phages were collected and dialyzed against buffered saline at room temperature. Then plaque-forming phages as well as *his*$^+$- and *try*$^+$-transducing particles were assayed. 0.1 ml-aliquots were soaked into glass-fiber filters (Schleicher & Schüll, No. 9), dried, precipitated with 10% TCA and washed with acetone. After drying, the filters were counted in toluene-scintillator in a Packard liquid scintillation spectrometer Mod. 3380.

This preparation for counting the radioactivity was performed also with the extract of prelabelled cells. For differential labelling of HT-mutants, the same procedure was carried out with the following exceptions: cell-labelling medium contained 10 µg cold thymidine/ml instead of 1 µg; phage-labelling medium contained 5 µC ^{32}P/ml instead of 1 µC, and lysates were grown in 1 ml-samples instead of 10 ml-samples.

Results

a) Isolation of Transduction Mutants: Possible Errors

The method for isolation of phage mutants with altered transduction abilities was described elsewhere in detail (Schmieger, 1971 b). We now know of several pitfalls in the procedure. Therefore it is desirable to describe briefly the salient feature of this technique. The procedure is as follows: Mutagenized phages are plated together with wild type indicator bacteria onto minimal-agar plates, so that up to 100 plaques appear after incubation. Thus, each plaque represents an individual lysate and the indicator bacteria are the donors for transduction. Then the wild type bacteria are killed by chloroform poured into the top lid of the plates. The plates should be incubated with chloroform at 37° C for not more than 1 hour. Then the top lids should be removed and incubation be continued for some hours to evaporate chloroform. If this period is too short too much chloroform remains dissolved in the agar layer and inhibits strongly the growth of transductants. After removing chloroform completely a concentrated suspension of stationary cells of an auxotrophic bacterial mutant is sprayed over the plates. The amount of sprayed cells is not critical. It is sufficient to be sure that the

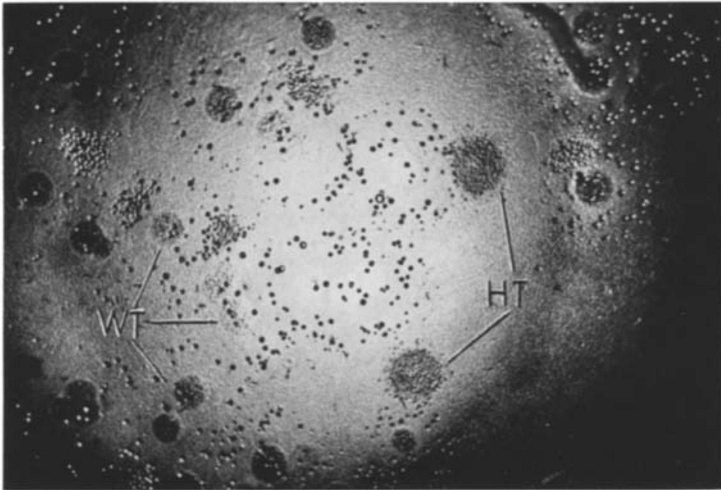

Fig. 1. *P22 H5*, a clear plaque mutant of *P22* with the wild type allele for transduction (*WT*), was mixed with *HT 12/4*, a mutant with increased transduction frequency, to demonstrate the technique. The phage mixture was plated with *LT2* onto M9-agar. After killing the indicator bacteria by chloroform, the plates were sprayed with *Try 8 (P22)*. *HT 12/4* plaques can easily be distinguished from *H5* by the large amount of transductant colonies covering them

plates look wet on the entire surface. Incubation is then continued for two days. During this time colonies of wild type transductants grow up around the plaques. The number of the transductants depends on the auxotrophic marker used. Plaques with considerably higher numbers of transductants can easily be distinguished from the normally transducing plaques, especially when using markers which cannot be transduced very frequently. Then the background frequency of transductants of non-mutated phages is low (Fig. 1). On the other hand, when using a well transducible marker like *his B22*, mutant plaques can easily be seen which have none or very few transductant colonies (Fig. 2).

Plaques of phages, supposed to be transduction mutants, are isolated and purified by replating. From three single plaques of each mutant a small sample is taken and conserved in 1.0 ml NB. Then the spraying procedure is repeated. After growing up the transductant colonies, one looks which one of the plaques from which a sample was taken shows still the altered transduction ability. From the corresponding conserved sample a lysate will be grown. The transduction frequency for a typical marker must be determined at multiplicities of infection lower than 10 and compared with wild type phage. Only after this last examination one can be sure to have a phage mutant altered in transduction frequency.

In both cases, i.e. increased as well as decreased numbers of transductant colonies, we isolated phages which show, even after repurification, the typical spraying picture. The T/P-ratios determined at low multiplicities of infection, however, are completely normal.

We presume that in those cases the phages produce an increased or decreased amount of lysozyme so that a higher or lower concentration will be present in

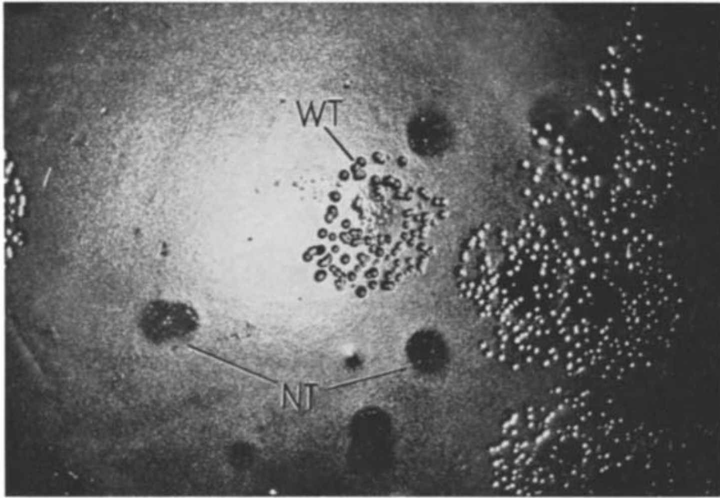

Fig. 2. A phage mixture was prepared from *P22 H5* (*WT* allele) and *NT 1/1*, a mutant with low transduction frequency derived from *H5*. The procedure was the same as described in Fig. 1 with the exception that *His HB22 (P22)* was sprayed as recipient. *NT*-plaques can easily be distinguished by the lack of transductant colonies

the plaque area. In the first case more potentially transduced cells would be killed than by wild type. In the second case more transduced cells could survive. In the following chapters only to those phage mutants will be referred as to be HT- or NT-mutants which exhibit altered transduction frequencies even at low multiplicities.

b) T/P-Ratios of NT- and HT-Mutants for Different Markers

It was of interest to know whether the frequencies of transduction in both types of mutants are changed only for the marker used for detection, or whether the frequencies for some or all markers are influenced. Some preliminary data were presented earlier (Schmieger, 1971b). To have a more complete picture, however, it was necessary to compare more mutants of the HT- and the NT-type and more recipient strains.

Table 1 shows a comparison of 5 independent NT-mutants with wild type phage *H5* (wild type with respect to the transduction ability). To have identical conditions, all lysates were adjusted to 2×10^{10} p.f.u./ml and transduction has been carried out with a multiplicity of 10. All recipient strains were lysogenic for wild type phage *P22* and used at stationary phase. The T/P-ratios were calculated as the number of complete transductants counted after two days per the number of plaque forming units on the plate. As can be seen in Table 1 it is impossible to get an uniform pattern of T/P-ratios. The marker used for detection of the NT-mutants was *his B22*. This marker is transduced by all mutants with a considerably lower rate. The reduction is at least to one half (*NT 6/2*) and in the best case (*NT 1/1*) two orders of magnitude. *try 8* also is affected in the same way. For *arg*, only *NT 1/1* and *NT 5/1* show somewhat reduced transduction

Table 1. Various T/P-ratios of NT-mutants in comparison to wild type phage

Phage	*his B22*+	*try-8*+	*arg*+	*cys E8*+	*met A22*+	*thr 47*+	*leu-39*+	*ara-9*+
H5	1.2×10^{-6}	3.3×10^{-7}	3.5×10^{-9}	$< 1.0 \times 10^{-9}$	1.0×10^{-9}	1.6×10^{-7}	5.1×10^{-8}	3.3×10^{-7}
NT 1/1	1.0×10^{-8}	1.8×10^{-8}	8.0×10^{-10}	$< 8.0 \times 10^{-10}$	$< 8.0 \times 10^{-10}$	1.2×10^{-7}	4.8×10^{-9}	3.2×10^{-7}
NT 5/1	4.6×10^{-8}	2.1×10^{-8}	8.7×10^{-10}	$< 8.7 \times 10^{-10}$	2.6×10^{-9}	1.3×10^{-7}	2.2×10^{-8}	2.7×10^{-7}
NT 6/2	5.0×10^{-7}	2.1×10^{-8}	8.9×10^{-9}	$< 1.1 \times 10^{-9}$	$< 1.1 \times 10^{-9}$	1.3×10^{-7}	5.9×10^{-8}	3.8×10^{-7}
NT 101/3	5.4×10^{-7}	1.0×10^{-7}	7.1×10^{-9}	1.4×10^{-8}	2.3×10^{-9}	1.7×10^{-7}	2.4×10^{-8}	4.7×10^{-7}
NT 104/3	8.5×10^{-8}	8.7×10^{-8}	2.6×10^{-8}	1.7×10^{-8}	$< 1.8 \times 10^{-9}$	1.9×10^{-7}	2.8×10^{-8}	6.2×10^{-7}

Table 2. Various T/P-ratios of HT-mutants in comparison to wild type phage

Phage	*his B22*+	*try-8*+	*arg*+	*cys E8*+	*thy T4/2*	*thr 47*+	*leu-39*+	*ara-9*+
H5	1.1×10^{-6}	3.9×10^{-7}	3.5×10^{-9}	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$	1.6×10^{-7}	5.1×10^{-8}	3.3×10^{-7}
HT 0/18/1	4.7×10^{-5}	1.4×10^{-5}	1.0×10^{-6}	1.0×10^{-6}	4.8×10^{-7}	2.4×10^{-6}	1.5×10^{-5}	1.3×10^{-5}
HT 6/1	6.9×10^{-5}	1.5×10^{-5}	3.6×10^{-6}	3.9×10^{-6}	4.6×10^{-6}	4.6×10^{-6}	4.0×10^{-5}	3.5×10^{-5}
HT 12/4	9.5×10^{-5}	1.6×10^{-4}	1.6×10^{-4}	1.0×10^{-4}	6.7×10^{-5}	5.7×10^{-5}	3.6×10^{-4}	3.4×10^{-4}
HT 15/2	3.2×10^{-5}	3.7×10^{-5}	5.8×10^{-6}	3.9×10^{-6}	2.8×10^{-6}	9.5×10^{-6}	2.1×10^{-5}	2.3×10^{-5}
HT 104/2	1.2×10^{-4}	3.5×10^{-5}	1.1×10^{-5}	1.2×10^{-5}	1.0×10^{-5}	1.1×10^{-5}	1.0×10^{-4}	7.7×10^{-5}
HT 105/1	1.4×10^{-4}	3.9×10^{-5}	6.9×10^{-6}	1.1×10^{-5}	5.0×10^{-6}	1.2×10^{-5}	1.1×10^{-4}	1.0×10^{-4}
HT 112/3	1.5×10^{-4}	2.8×10^{-5}	7.3×10^{-6}	1.1×10^{-5}	7.1×10^{-6}	1.0×10^{-5}	1.0×10^{-4}	1.0×10^{-4}

frequencies; the other mutants are in the range of wild type or seem to be even better (*NT 104/3*). A similar picture can be seen for the markers *cys E8* and *met A22*. The markers *thr-47*, *leu-39* and *ara-9* which are close together on the chromosomal map of *S. typhimurium* and from which *leu* and *ara* can be transduced by the same fragment (Sanderson, 1970) are not affected at all.

There seem to be contradictions between the data already published and the date here presented regarding T/P-ratios for the transductions *NT 1/1* × *His HB22*, *NT 101/3* × *His HB22* and *NT 1/1* × *Try 8*. The rates now do not deviate so extremly from the wild type values as the earlier published data. This can be explained by different multiplicities of infection. We learned that the T/P-ratios of many — if not all — NT-mutants are more dependent on the multiplicity of infection than wild type phages. This dependence cannot yet be explained.

The T/P-ratios for several HT-mutants were determined in the same way, transducing the same recipient strains. Only *Met A22 (P22)* was replaced by *Thy T4/2 (P22)*. All procedures were carried out as described for the NT-phages, with the exception that a moi of 1 was maintained in all adsorption mixtures (Table 2). Here the pattern is much more uniform. We see that the T/P-ratios of all mutants tested for a single marker are very similar with a few exceptions. However, it is very interesting that the ratios are also nearly identical when comparing the values of all recipients for a certain HT-mutant. This means that the characteristic widely ranging differences of the T/P-ratios exhibited by wild type phages disappear and reach optimal values. Therefore the increase of the transduction frequencies is more expressed where the frequencies are lower in wild type lysates. So the T/P-ratio can be increased by more than five orders of magnitude (see *HT 12/4* and *Cys E8*).

c) Are the NT- and HT-Effects due to Different Numbers of Transducing Particles in the Lysates?

The various T/P-ratios (Table 1 and 2) are calculated from the numbers of complete transductant colonies arising from HT- and NT-transduction experiments. However, with these values only, it cannot be decided whether the actual number of transducing particles in a lysate is different from that in a lysate of wild type phages.

It may also be that the probability of integration of the transduced chromosomal fragment into the bacterial recipient chromosome has changed in some way. Since non-integrated fragments lead to abortive transductants (Ozeki, 1956; Stocker, 1956), this kind of alteration should result in altered relations between the number of complete and that of abortive transductants. NT-phages should produce more abortive microcolonies causing a smaller number of complete transductants. HT-phages, in contrast, should produce the higher amount of complete transductants by a more frequent integration of fragments. At least for HT-phages this explanation was improbable since the ratio abortive/complete transductants normally varies around 10 and rarely exceeds a value of 20 (Ozeki, 1959). Therefore, increases of T/P-ratios by two orders of magnitude and more could not be explained even supposing that all fragments of a certain marker

Table 3. Ratio abortive/complete transductants

Phage	Transduced markers		
	his B22$^+$	*try 8*$^+$	*leu 39*$^+$
H5	17	5.7	1.1
HT12/4	12.8	8.0	1.4
NT 1/1	8.2	5.6	1.5

Transductions were carried out as usual. Dilutions were performed in NB to ascertain identical enrichment on all selective plates. Then aliquots were distributed on the surface of M9-agar-plates. Plates were incubated for two days at 37° C, complete transductants counted and plates incubated for another day. Then abortive transductants were counted using a microscope with 10–20× magnification. The ratios of the corresponding numbers of abortive and complete transductants were calculated and are shown in the table.

become integrated into the host chromosome. Nevertheless transduction experiments with wild type phages, HT- and NT-mutants were performed and complete as well as abortive transductants were counted (Table 3). The ratios for the same markers do not differ much between the mutant and wild type phages. Therefore, it clearly turns out that an increased or decreased probability of integration cannot account for the larger or smaller numbers of complete transductants.

Double labelling experiments were performed to demonstrate directly the altered numbers of transducing particles in relation to the number of infective phages. Transducing particles of *P22* are known to contain DNA molecules of which about 90% originate from the bacterial chromosome synthesized before infection (Schmieger, 1968, 1970). On the other hand, infective phages contain only DNA synthesized after infection or parental DNA (Backhaus and Schmieger, to be published), but no detectable amount of DNA material of the bacterial cell (Schmieger, 1971a). This allows a selective labelling of transducing particles with tritium by infecting bacteria prelabelled with ^{3}H-thymidine. In a forthcoming paper it will be shown that these conditions are true also for HT-mutants and that the differential labelling technique may be applied also to them. Plaqueforming phages of the same lysate were labelled with ^{32}P by transferring the infected cells into a medium containing cold thymidine and radioactive phosphorus. After careful purification of such a differentially labelled lysate ^{3}H-counts represent the total of transducing particles irrespective of the markers carried and ^{32}P-counts signify infective particles, possible defective phages included. The 10% contribution of phage DNA present in transducing particles (Schmieger, 1970) should also have the ^{32}P-label. It can be neglected, however, on account of the small amount of transducing particles.

By this method we prepared double-labelled lysates of wild type phages and several NT- and HT-mutants. Aliquots of the purified phage suspensions were counted and the ratio of ^{3}H/^{32}P determined. To detect possible variations in the amount of defective phages, which could influence the T/P-ratio, the ratio of ^{32}P-counts per plaque forming unit has also been calculated. Tables 4 and 5 show

Table 4. Differentially labelled lysates of wild type phage and NT-mutants

Phage	Titer	^3H-cpm	^{32}P-cpm	$\frac{^{32}P\text{-cpm}}{\text{p.f.u.}}$	$\frac{^3H\text{-cpm}}{^{32}P\text{-cpm}}$
H5	7.6×10^{10}	1280	5229	6.9×10^{-7}	0.244
NT 1/1	6.7×10^{10}	179	4570	6.8×10^{-7}	0.039
NT 6/2	1.2×10^{10}	23	595	5.0×10^{-7}	0.039
NT 101/3	9.9×10^{9}	82	810	8.2×10^{-7}	0.101
NT 5/1	1.9×10^{9}	82	863	4.5×10^{-7}	0.095

Table 5. Differentially labelled lysates of wild type phage and HT-mutants

Phage	Titer	^3H-cpm	^{32}P-cpm	$\frac{^{32}P\text{-cpm}}{\text{p.f.u.}}$	$\frac{^3H\text{-cpm}}{^{32}P\text{-cpm}}$
H5	1.6×10^{10}	945	57396	3.8×10^{-6}	0.016
HT 12/4	3.9×10^{7}	584	191	4.9×10^{-6}	3.058
HT 15/2	1.6×10^{8}	183	916	5.7×10^{-6}	0.200
HT 104/2	5.1×10^{7}	74	400	7.8×10^{-6}	0.185
HT 105/1	1.9×10^{8}	355	1151	6.1×10^{-6}	0.308
HT 6/1	9.1×10^{8}	992	3614	4.0×10^{-6}	0.274
HT 112/3	4.2×10^{8}	516	2362	5.6×10^{-6}	0.218

the results for NT-mutants and HT-mutants respectively. To favour the detection of the expected fewer ^3H-counts in NT-lysates, the specific activity of ^{32}P in these preparations was kept lower and the specific ^3H-activity higher. Therefore the results in the two tables can only be compared to their *H5*-value which resulted from the respective labelling conditions. HT- and NT-values cannot be compared directly.

It should also be pointed out that the low titers of the mutant phages, especially of the HT-series are not a characteristic of the mutants. We worked with very small lysates and purified them in a stepwise CsCl-gradient as described in Material and Methods. On account of the small number of phages they could not be seen as a band and we lost therefore particles when harvesting them. Only *HT 12/4* and *NT 5/1* give lower titers under normal growth conditions.

In Table 4 we see significant drops in the ratios $^3H/^{32}P$ of NT-mutants compared with *H5*. The drop is not so drastic for *NT 5/1* and *NT 101/3*. The titers of plaque-forming phages in the original lysates were similar and the ratios ^{32}P cpm/p.f.u. are nearly identical. Therefore the drop of the $^3H/^{32}P$-ratios cannot be explained by higher count rates of ^{32}P due to exceptional overproduction of plaque-forming phages and constant numbers of transducing particles. The ratios can only be explained by smaller count rates of ^3H which means reduced numbers of transducing phages in NT-mutant lysates.

Table 5 shows the results of differentially labelled lysates of HT-mutants compared with wild type. In this case the $^{3}H/^{32}P$-ratios are markedly increased, from 12-fold (*HT 104/2*) to nearly 200-fold (*HT 12/4*). Before purification the HT-lysates had also similar titers as wild type, except *HT 12/4* which was about one order of magnitude lower. This means that a possibly reduced formation of infective phages and a constant number of transducing particles cannot account for the increased $^{3}H/^{32}P$-values.

One could have argued when interpreting the T/P-ratios of Table 2 that possibly there is a large number of defective phages which cannot be detected by the plaque-assay but would account for the high T/P-ratios of Table 2. However, these phages would contribute to the ^{32}P-count rates and should be seen in deviating ratios ^{32}P cpm/p.f.u. As can be seen these values are nearly identical; a significantly larger number of defective phages in HT-mutant is, therefore, excluded.

So, it is clear that the increased $^{3}H/^{32}P$-values of HT-mutants are due to more transducing particles in the lysate.

d) How Much DNA of an Infected Bacterial Culture Becomes Converted to Transducing Fragments?

When growing the differentially labelled lysates (presented in Table 4 and 5), a sample of the ^{3}H-labelled bacterial culture was also prepared for counting at the time of phage infection, to have a measure for total DNA present in the infected culture in the moment of infection in terms of ^{3}H-counts. Knowing the titers of plaque-forming particles before and after purification one could calculate how many ^{3}H-counts were present in an equal volume of the original crude lysates of the phage-mutants. However, one has to assume that the titer of transducing particles changes in the same way as that of plaque-forming particles during purification of the lysate. This assumption is justified by all known physical data of transducing and plaque-forming phages (Schmieger, 1968). This calculation is necessary, since it is not possible to measure the ^{3}H-counts in transducing particles in the crude lysate in a direct way. The free bacterial DNA of the lysed cells which carries also the ^{3}H-label contaminates the lysate.

Setting the ^{3}H-counts of the uninfected culture equal to 100%, the ^{3}H-counts in transducing phages, computed for the crude lysate, would indicate the percentage of bacterial DNA packed into transducing particles. Tables 6 and 7 present the values for the same phage mutants appearing also in the two foregoing tables.

One can see that in both experiments wild type phage incorporates a very similar amount of bacterial DNA into transducing particles: 0,7%, 1,0%, resp. Again it turns out that the NT-mutants indeed produce a smaller number of transducing particles. HT-phages, however, exceed the value of wild type up to nearly 20-times. Using different growth conditions we could show that mutant *HT 12/4* is able to convert up to about 50% of bacterial DNA to transducing fragments.

Table 6. Quantitative estimation of host DNA converted to transducing fragments by wild type phage and NT-mutants

Phage	^{3}H-cpm in 0.1 ml purified lysate	^{3}H-cpm in 0.1 ml crude lysate	% of total ^{3}H-cpm in 0.1 ml culture (100% = 75050 cpm)
H5	1280	487	0.7
NT 1/1	179	59	0.08
NT 6/2	23	4.6	0.006
NT 101/3	82	30	0.04
NT 5/1	82	30	0.04

Table 7. Quantitative estimation of host DNA converted to transducing fragments by wild type phage and HT-mutants

Phage	^{3}H-cpm in 0.1 ml purified lysate	^{3}H-cpm in 0.1 ml crude lysate	% of total ^{3}H-cpm in 0.1 ml culture (100% = 34854 cpm)
H5	945	349	1.0
HT 12/4	584	4792	13.7
HT 15/2	183	1487	4.3
HT 105/1	354	2987	8.6
HT 6/1	992	6208	17.8
HT 112/3	516	3070	8.8

e) Dominance of HT- and NT-Properties

Since it is clear that the ability to form more or less transducing particles is a genetically determined property of the phage, it was of interest to see if this property is dominant in mixed infections or not. Therefore, donor cells were infected simultaneously with *H5* and *NT 1/1* and with *H5* and *HT 12/4* respectively at varied multiplicities of infection. The resulting lysates were assayed for plaque-forming particles and transduction of one or two bacterial markers. The results of the NT-experiment are shown in Table 8. One can see that the titers of the resulting mixed lysates do not vary markedly. Concerning the T/P-ratios of his^+-particles they show in all mixed lysates smaller values than obtained with wild type (No. 1). However, in lysates 6 and 7 with higher wild type input than NT, the T/P-ratios are already close to pure wild type transduction. The T/P-ratios of lysates 3 to 5 indicate that there is a considerable influence of the NT-property.

A similar result turns out for *HT 12/4–H5* mixed infections. In this case it was possible to distinguish the HT-plaques from *H5* morphologically, and we could assay the transduction ability for a rarely transducible marker, *leu 39*. Table 9 shows the results. We see a significant influence of the different multiplicities of infection on the development of phages. Both phages, wild type as

Table 8. Dominance of *NT 1/1*

No. of lysate	moi w.t.	moi *NT 1/1*	p.f.u./ml	T/P_{his^+}
1	3	—	5.8×10^{10}	1.4×10^{-6}
2	—	3	5.4×10^{10}	2.6×10^{-8}
3	3	3	7.8×10^{10}	5.0×10^{-7}
4	3	21	6.6×10^{10}	1.5×10^{-7}
5	3	30	4.7×10^{10}	1.9×10^{-7}
6	21	3	6.1×10^{10}	9.4×10^{-7}
7	30	3	7.0×10^{10}	8.0×10^{-7}

Table 9. Dominance of *HT 12/4*

No. of lysate	moi *H5*	moi *HT 12/4*	p.f.u. *H5* per ml	p.f.u. *HT 12/4* per ml	T/P_{leu^+} for total titer
1	3	—	6.7×10^{10}	—	8.5×10^{-8}
2	—	3	—	1.1×10^{10}	2.9×10^{-4}
3	3	3	2.9×10^{10}	1.6×10^{10}	3.0×10^{-5}
4	3	21	3.9×10^{9}	1.5×10^{10}	1.2×10^{-4}
5	3	30	4.6×10^{9}	1.7×10^{10}	1.3×10^{-4}
6	21	3	5.4×10^{10}	4.9×10^{9}	1.5×10^{-6}
7	30	3	5.3×10^{10}	2.4×10^{9}	7.5×10^{-7}

well as *HT 12/4*, inhibit the growth of the other phage when infecting at higher multiplicity. This complicates the interpretation of the experiments. It is very probable that the same effect is true in the NT-experiment; however, there is no possibility to distinguish *NT 1/1*- and *H5*-plaques.

In Table 9 the T/P-ratios were calculated for the *leu*$^+$-marker disregarding the phage types; this means that the total titer of plaque-forming phages was taken for the P-value. One can see that the T/P-ratios are much higher in all mixed lysates than in the wild type lysate, included No. 6 and 7 which show higher values despite the higher inputs of *H5*.

On the other hand T/P-ratios of mixed lysates are lower than expected if they were mixed in the respective ratios of pure wild type and *HT 12/4*. We calculated the complete transducing particles that can be expected for the lysates according to their individual titers (Table 9) and using the characteristic T/P-ratios of the pure lysates No. 1 and 2. The sum of the expected *H5*- and *HT 12/4*-transducing particles and the actual number of complete transducing particles found in the experiment are shown in Table 10. These data show clearly that *HT 12/4* cannot exhibit its characteristic T/P-ratio completely although it increases strongly the number of transducing particles in mixed lysates.

Table 10. Comparison of the expected and found numbers of transducing particles (t.p.) for *leu*+ in mixed lysates of *H5* and *HT 12/4*

No. of lysate	total *leu*+ t.p. calculated	total *leu*+ t.p. found	found/ calculated
1	5.7×10^3	5.7×10^3	—
2	3.2×10^6	3.2×10^6	—
3	4.6×10^6	1.3×10^6	0.28
4	4.4×10^6	2.3×10^6	0.52
5	4.9×10^6	2.8×10^6	0.57
6	1.4×10^6	1.0×10^5	0.07
7	7.0×10^5	4.1×10^4	0.06

Discussion

By a simple method we were able to isolate mutants of phage *P22* which exhibit alterations in their abilities for generalized transduction (Schmieger, 1971 b). They show increased or decreased frequencies for different markers.

By different methods we could show that this is due to larger or smaller numbers of transducing particles present in the mutant lysates. At first, it could be excluded that the probability of integration of the transduced fragments into the recipient chromosome has changed. Then, by differentially labelling the DNA of transducing and plaque-forming particles with radioisotopes, we could demonstrate directly that the actual numbers of particles are different from wild type phage. To permit the application of this technique to the transduction mutants one had to assume that the physical characteristics of the transducing particles of such mutants are the same as in wild type and that they are formed by the same process. It will be shown in a forthcoming paper that this assumption is justified.

The labelling experiments already showed that NT-mutants do not give results as marked as HT-phages do. In two cases their ratios of transducing to plaque-forming phages (T/P-ratio) were decreased only by a factor 2.5, while the other mutants deviate by a factor of 6. HT-mutants show increases of the T/P-ratio of at least a factor of 11 compared with wild type.

The same weak responses of NT-mutants turned out when studying their transduction frequencies for different markers more in detail.

It turned out that in NT-mutants with decreased transduction frequencies not only the marker used for detection of the mutant is affected. A smaller T/P-ratio can also be observed for other markers. However, the reductions are not of the same dimensions. We observe also that certain markers are not at all affected. To explain this we should know more about the functions involved in the process of formation of transducing particles.

The HT-mutants, however, show increased transduction frequencies for all markers tested. But not all markers are increased by the same rate. The T/P-

ratios of wild type lysates vary between 10^{-6} to $<10^{-9}$ for different markers following our transduction procedure. It is important to note that HT-mutants transduce the same markers with a few exceptions with a ratio between 10^{-4} and 10^{-5}. This means that markers which can rarely be transduced by wild type *P22* are mostly affected in HT-mutants. The reason is still unknown but one may speculate that possibly there is a step in particle formation which occurs with certain preferences. The specificity of this reaction may be changed or even getting lost completely in HT-mutants. Ozeki's results (1959) that transducing fragments carrying the same marker have identical endpoints indicate also that there is a selective step. However, instead of end*points* one should speak about preferred end*regions* according to other authors who found variations in the fragments (Enomoto, 1965; Pearce *et al.*, 1965; Roth *et al.*, 1965). It may well be — mapping experiments (publication under preparation) support this idea — that the product of the HT-gene is a nuclease which cuts the host DNA at certain points. Its action should depend on the recognition of certain base groups. Supposing the loss of this requirement of recognition in HT-phages the nuclease would act at any point with the same or a similar probability. By this way, all transducing particles would be formed with similar frequencies. Further experiments will reveal whether such an enzyme action can account for the mechanism of fragmentation.

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