# LOCALIZATION OF STREPTOLYDIGIN RESISTANT MUTATION IN E. COLI CHROMOSOME AND EFFECT OF STREPTOLYDIGIN ON T2 PHAGE DEVELOPMENT IN stl-r AND stl-s STRAINS OF E.COLI

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SUMMARY

The order of mutations affecting RNA polymerase is thi - rif- - stl-argH. Transduction experiments revealed high frequency of recombination between rif and stl markers (about 50 per cent). Streptolydigin as well as rifampicin inhibits phage T2 development in the host cells sensitive to the drugs but not in rif-r stl-r host cells.

Three different types of mutations have recently been described affecting E.coli RNA polymerase: (I) temperature-sensitive mutations, one of which - tsX - was found to impair the attachment of the enzyme to DNA template (1,2), (2) rif-r mutations leading to the enzyme resistance to rifampicin which is known to inhibit initiation of RNA synthesis (2-6), (3) stl-r mutations resulting in the enzyme resistance to streptolydigin which blocks elongation of RNA chains (7,8). All these mutations were mapped between argH and thi markers on the E.coli chromosome. The following sequence of markers was described: thi - rif - tsX - metB (2). In this work the mapping of stl marker was performed relative to the position of thi and rif. It is known that at least part of bacterial RNA polymerase participates in T-even phage development, although modification of the enzyme takes place after infection (9,10). Rifampicin has been previously shown to inhibit the development of DNA bacteriophages in bacteria sensitive but not resistant to the drug (11-13). In this work the effect of streptolydigin on the T2 phage development in stl-r and stl-s host cells has been also studied.

## Methods and results

Isolation and properties of stl-r mutants. Bacterial strains used were all derivatives of E.coli K-12. For isolation of stl-r mutants E.coli strain RTS 522 derivative of P678 permeable to streptolydigin was used (7). As we failed to isolate stl-r mutants with altered RNA polymerase among spontaneous stl-r mutations, UV irradiation ( ~ 20% survival) was performed. UV-irradiated cultures were aerated in broth for 6 hours at 37° and then plated on agar containing 100 µg/ml of streptolydigin for selection of stl - resistant colonies. RNA polymerase activity in the presence or absence of the drug was

Effect of streptolydigin on RNA synthesis in extracts of different stl-r E. coli mutants

| No. of stl-r mutant | Counts /100"/ sample without stl | Counts /100"/ sample with stl | % inhibition | No. of stl-r mutant | Counts /100"/ sample without stl | Counts /100"/ sample with stl | % inhibition |
|---|---|---|---|---|---|---|---|
| 1 | 4.215 | 427 | 90 | 16 | 2.169 | 1.598 | 26 |
| 2 | 6.106 | 1.214 | 80 | 17 | 4.911 | 2.349 | 52 |
| 3 | 4.126 | 123 | 97 | 18 | 11.789 | 10.093 | 14 |
| 4 | 3.170 | 1.257 | 60 | 19 | 4.550 | 502 | 99 |
| 5 | 6.118 | 169 | 97 | 20 | 6.940 | 504 | 93 |
| 6 | 4.988 | 195 | 96 | 21 | 8.476 | 602 | 93 |
| 8 | 3.607 | 2.248 | 38 | 22 | 10.414 | 591 | 94 |
| 14 | 6.338 | 5.255 | 17 | 23 | 9.821 | 617 | 94 |
| 15 | 9.010 | 237 | 97 | stl-s RTS 522 | 4.948 | 353 | 93 |

Extract preparation, precipitation of DNA and $^{14}$C-UTP-incorporation analysis were performed according to Chamberlin and Berg (1962). T2-DNA was added. Final concentration of streptolydigin (stl) was 80 µg/ml.

determined in extracts after the removal of DNA with streptomycin or in partly purified preparations /stage III according to Chamberlin and Berg (14)/. The results are summarized in Table 1.

Ten out of seventeen stl-r mutants have streptolydigin - sensitive RNA polymerase. The phenotype of these mutants apparently is a result of alteration of cell wall permeability to the drug. RNA polymerase of the rest of mutants reveal different levels of resistance to streptolydigin. It should be emphasized that up to now no stl-r mutation has been found with RNA polymerase completely resistant to streptolydigin. In contrast mutations to rifampicin - resistance often demostrate complete resistance to high concentration (100-200 μg/ml) of the drug. This difference may reflect the different modes of inhibitory action of the two antibiotics. RNA polymerase of all the stl-r mutants tested retains sensitivity to rifampicin characteristic to the original stl-s strain.

Rif-r spontaneous mutation in stl-r strain was isolated on the plates containing 100 μg/ml of rifampicin. As it was shown by biochemical analysis introduction of spontaneous rif-r mutations into stl-r strain did not modify the resistance of RNA polymerase to streptolydigin. It is evident from the recombination frequency between rif and stl markers that the two mutations can be easily combined in the same genome (Table 2). In contrast it was previously shown in transduction or conjugation experiments (15) that combination of other types of mutations affecting RNA polymerase, namely tsX and rif-r-1 is lethal.

Mapping of stl-r-8 mutation on E. coli chromosome. To locate stl-r mutation on the E.coli chromosome phage P1 mediated transduction have been carried out. The bacterial strains used and the results of transduction are presented in Table 2. Frequency of cotransduction of rif-r or rif-s markers with the thi$^+$ marker varies from 64,6 to

Table 2

Transduction mapping of stl-r-8 mutation on E. coli chromosome

| Exp. No. | Donor | Recipient | Selective marker | Recombinant types | Transductants in total | Number of recombinants | % co transfer with thi+ rif+ | % co transfer with thi+ stl+ | stl/rif x 100x) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | R25 thi+ rif-r stl-s | RTS8 thi rif-s stl-r | + thi | rif-r stl-s<br>rif-s stl-r<br>rif-r stl-r<br>rif-s stl-s | 99 | 32<br>35<br>32<br>0 | 64,6 | 32,7 | 50,0 |
| 2 | -"- | -"- | thi+ | rif-r stl-s<br>rif-s stl-r<br>rif-r stl-r<br>rif-s stl-s | 137 | 52<br>38<br>47<br>0 | 72,2 | 37,2 | 47,4 |
| 3 | R1 thi+ rif-s stl-s | RTS 105 thi rif-r stl-r | thi+ | rif-s stl-s<br>rif-r stl-r<br>rif-s stl-r<br>rif-r stl-s | 118 | 43<br>32<br>43<br>0 | 72,8 | 36,4 | 50,0 |

Transduction was performed according to Lennox (16). R25 - derivative of R$\frac{1}{1}$ with rif-r-1 mutation introduced by conjugation (1). RTS8 - derivative of RTS 522 with UV-induced resistance of RNA polymerase to streptolydigin. RTS 105 - derivative of RTS 8 stl-r-8 with spontaneous RNA polymerase resistance to rifampicin.

x) Per cent of transductants with recipient stl-marker among transductants with rif-marker of donor strain.

72,8 per cent. The *stl-s* merker is cotransducible with the *thi*$^+$ marker at a frequency of 32,7 to 37,2 per cent. Among the *thi*$^+$ transductants tested no recombinants have been found carrying the *stl-s* donor marker and the *rif*-marker of recipient strain. These results show that *rif* is located closer to the *thi* than the *stl*-marker. In addition, transduction of AB 1450 *rif-s stl-s argH* with a stock of P1 phage grown on strain RTS 18 *rif-r stl-r argH*$^+$ was performed. All the *argH*$^+$ *rif-r* transductants tested revealed RNA polymerase resistant to streptolydigin. Basing on these results the order of the markers seems to be the following *thi-rif - stl - argH*.

As it can be seen from Table 2, *stl* and *rif* markers were separated by recombination with the frequency of 47,5 - 50 per cent, which indicates that these two mutations are localized relatively far from each other and probably affect different genes. This suggestion however needs further confirmation.

*The effect of streptolydigin on phage T2 development*. Strain RTS 522 was found to be poorly infected with T2 phage which may be partly attributed to T1$^r$ (*ton*) mutation which cause change of the cell wall. Mating of *Hfr* 30S0 *thr*$^+$ *leu*$^+$ *ton*$^+$ *str-s thi*$^+$ and F$^-$ RTS 522 *thr leu ton str-r thi* was carried out and recombinant RTS 522-15 *thr*$^+$ *leu*$^+$ *ton*$^+$ *str-r thi* was selected with restored ability to be infected with phage T1. Strain RTS 522-15-1 *stl-r rif-r* was obtained by the one step transduction of the *rif-r* and the *stl-r* markers into the strain RTS 522-15. RNA polymerase from RTS 522-15-1 was completely resistant to 100 μg/ml of rifampicin and demonstrated only 25 per cent inhibition by 100 μg/ml of streptolydigin while the enzyme from original RTS 522-15 strain was practically completely inhibited by these concentrations of the drugs.

Experiments on the effect of the antibiotics on phage T2 reproduction in sensitive and resistant strains are summarized in Table 3.

Table 3

Effect of rifampicin and streptolydigin on the T2 phage development in *E. coli* strains RTS 522-15 *rif-s stl-s* and RTS 522-15-1 *rif-r stl-r*.

| Drug added | Time of addition (min after adsorption) | Host bacteria | | | |
|---|---|---|---|---|---|
| | | rif-s stl-s | | rif-r stl-r | |
| | | Phage yeild per cell | % | Phage yeild per cell | % |
| | | Exp.I | | | |
| - | - | 213,8 | /100/ | 94,3 | /100/ |
| Rif. | 0 | 2,2 | 1,0 | 67,3 | 71,6 |
| Rif | 10 | 12,6 | 5,9 | | |
| Stl | 0 | 2,5 | 1,2 | 74,2 | 78,7 |
| Stl | 10 | 9,5 | 4,5 | | |
| | | Exp. II | | | |
| - | - | 34,5 | /100/ | 54,0 | /100/ |
| Rif | 0 | 0,6 | 1,7 | 53,2 | 98,5 |
| Rif | 10 | 0,7 | 1,9 | | |
| Stl | 0 | 1,7 | 4,8 | 40,9 | 75,8 |
| Stl | 10 | 1,4 | 4,0 | | |

T2 phage was adsorbed on cells for 7 min at 37° in Exp.I and at 30° in exp. 2 with a multiplicity of 0,004. Then 0,02 ml samples were transfered into 1 ml of broth with or without 100 μg/ml of drug or supplied with T2-antiserum for estimation of the amount of infected cells. The latter were incubated for 7 min. Control samples and samples with the drugs were incubated for 60 min. Some samples were supplied with drugs after 10 min. incubation and than incubated for 50 min more. Incubation was stopped by cooling; then the samples were diluted and plated on *E. coli B* lawn.

It may be seen that phage reproduction was completely inhibited by addition of rifampicin and streptolydigin in sensitive host bacteria.

Both early and late stages of latent period were sensitive to the drugs. In contrast double rif-r stl-r mutant strain did support phage development in the presence of streptolydigin and rifampicin.

These results demonstrate that at least two parts of RNA polymerase involved in RNA synthesis during early and late stages of T2 phage development are coded by bacterial but not by viral genome.

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