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Mapping of Two Transcription Mutations (*tlnI* and *tlnII*) Conferring Thiolutin Resistance, Adjacent to *dnaZ* and *rho* in *Escherichia coli*

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Summary. Two mutations in *Escherichia coli* conferring resistance to the transcription initiation inhibitor, thiolutin, have been mapped. One of these mutations (*tln-I*) maps at 10.2 min on the genetic map and is cotransducible with *dnaZ* at a frequency of approximately 50%. The other mutation (*tln-II*) maps between *metE* and *ilvD*, probably close to *rho*, and is cotransducible with *ilvD* at a frequency of approximately 65%. The presence of both the mutations in the same cell confers resistance to thiolutin in minimal medium. Either one of them alone renders the cell 'conditionally auxotrophic' in the presence of the drug. The implications of these findings are discussed in relation to the mode of action of the thiolutin sensitive factors in transcription.

Introduction

We have been studying the mode of action of and genetics of resistance to thiolutin (6-acetamido-4-methyl-1,2-dithiolo(4,3b)pyrrol-5(4H)-one), a sulfur-containing broad spectrum antibiotic isolated from *Streptomyces luteoreticulii* (Celmer and Solomon 1955). This drug inhibits RNA synthesis and the activity of RNA polymerases I, II and III in yeast (Tipper 1973). It was believed to inhibit the chain elongation step of RNA synthesis in *E. coli* (Khachatourians and Tipper 1974a, b). Our studies on the mode of action of thiolutin (Sivasubramanian and Jayaraman 1976) suggested that chain initiation could be inhibited by the drug. Although thiolutin inhibits RNA synthesis in *E. coli*, it has been observed (Sivasubramanian and Jayaraman 1976; Tipper, personal communication) that RNA polymerase activity is insensitive to the drug in vitro. Previous studies (Tipper 1973) showed that thiolutin might not act via the template. Thus this drug is a novel transcription inhibitor which acts through neither the template DNA nor RNA polymerase. Possibly, the inhibition of RNA synthesis in vivo is brought about through interaction with one or more of the accessory transcription factors which might control aspects such as promotor selection or conformation of RNA polymerase.

We have isolated mutants of *E. coli* resistant to thiolutin and studied their properties (Sivasubramanian and Jayaraman 1976). In this paper we present data showing map positions of two mutations conferring thiolutin resistance (*tlnI* and *tlnII*), near *dnaZ* and *rho* respectively and discuss implications of these findings.

Materials and Methods

a) Bacterial Strains. All bacterial strains used in this study are derivatives of *E. coli* K12 and are listed in Table 1. The mutations *tln-I* and *tln-II* were originally designated as TL'I and TL'II respectively (Sivasubramanian and Jayaraman 1976). The nomenclature has been changed in conformity with the Demerec system. Figure 1 shows the location of markers, points of origin and direction of DNA transfer of the various *Hfr* strains used.

b) Chemicals. Thiolutin was a gift from Nathan Belcher, Pfizer Inc., Groton, Conn. (USA). Solutions of thiolutin were freshly prepared in dimethyl sulphoxide at a concentration of 10 mg/ml and used at a final concentration of 25 µg/ml in liquid media and 30–40 µg/ml in solid media.

c) Growth Media. The rich medium used was Luria broth. R broth (Miller 1972) was used for preparing Pl lysates. A variation of the Davis and Mingioli medium (Miller 1972) was used for minimal medium. Nutritional supplements (L-aminoacids, purines, pyrimidines etc.) were added at 20–30 µg/ml. Thiamine hydrochloride was used at 10 µg/ml. Minimal selection plates contained 100 µg/ml streptomycin sulphate to counterselect against *Hfr* cells.

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Table 1. Bacterial strains

Strain	Sex	Genotype	Source or Reference
NSJ 74	Hfr KL 16	*tln-I*	Sivasubramanian and Jayaraman (1976)
NSJ 75	Hfr KL 16	*tln-I, tln-II*	Sivasubramanian and Jayaraman (1976)
NSJ 76	F^-	*tln-I*	NSJ 74 × CSH 57b
NSJ 77	F^-	*tln-I, tln-II*	NSJ 76 × NSJ 75
NSJ 78	F^-	*tln-II*	NSJ 77 × KL 226
CSH 57b	F^-	*ara, leu, lacY, purE, gal, trp, his, argG, malA, rpsL, xyl, mtl, ilv, metA, thi*	Cold Spring Harbor Collection
χ478	F^-	*ara, leu, azi, lacZ, proC, purE, trp, lysA, rpsL, xyl, mtl, metE, thi*	
AX 727	F^-	*dnaZ, thi, lac, rpsL*	James Walker
AT 2538	F^-	*thi, pyrE, argE, his, proA, thr, leu, mtl, xyl, ara, gal, lacY, rpsL, supE44*	C G S C[a]
AB 3505	F^-	*proA, trp, his, ilvD, metE, argH, lacY, lacZ, galK, xyl, mtl, malA, supE44*	C G S C
AB 259	HfrH	*thi, relA*	C G S C
KL 14	Hfr	*thi, relA*	C G S C
KL 16	Hfr	*thi, relA*	C G S C
KL 25	Hfr	*supE42*	C G S C
KL 208	HfrB7	*relA*	C G S C
KL 209	HfrJ4	*thi, malB, supE44*	C G S C
KL226	HfrC	*relA, tonA*	C G S C
KL228	Hfr	*thi, leu, gal, lacY, supE44*	C G S C
HfrB4	HfrB4	*metB, relA*	C G S C
HfrB8	HfrB8	*metB, relA*	C G S C

[a] CGSC = Strains received from Dr. B.J. Bachmann, Coli Genetic Stock Center, Yale University School of Medicine, New Haven, Conn., USA

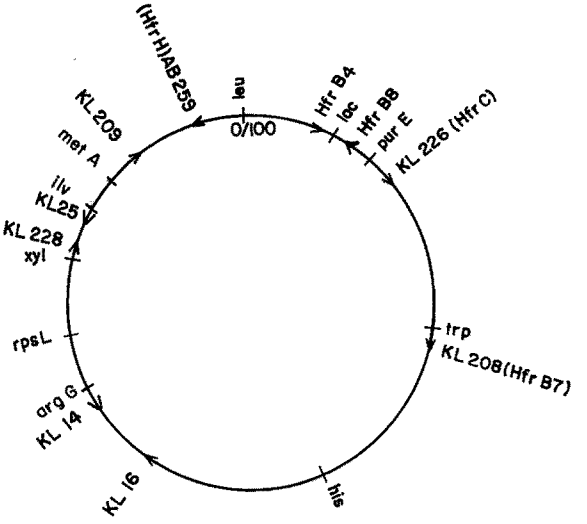

Fig. 1. The location of markers, points of origin and direction of DNA transfer of the various *Hfr* strains used. Not drawn to scale

d) Conjugation Experiments. Conjugation was performed by conventional methods. For interrupted mating, the *Hfr* and F^- cells were grown to mid-log phase and mixed at a ratio of 1:10 respectively. Mating was performed in 10 ml volumes in 250 ml Erlenmeyer flasks at 37 °C without shaking. 0.5 ml samples were withdrawn when necessary, diluted tenfold in saline and vortexed for a minute to interrupt mating. Whenever appropriate, aliquots of the samples were inoculated into 10 ml of broth containing streptomycin and grown overnight to allow segregation of markers. For uninterrupted mating experiments, the mating was continued for 90 min.

e) Procedure for Scoring Thiolutin Resistance. Sensitivity or resistance to thiolutin was scored on either one or both of the following media: (a) Minimal-drug plates, containing the requisite nutritional supplements and the drug; (b) Minimal-broth-drug plates, containing the requisite nutritional supplements, Luria broth (10 ml/100 ml) and the drug. In crosses involving thiolutin-resistant *Hfr*s, the mated culture was segregated in streptomycin containing broth and thiolutin – resistant recombinants were scored on minimal-broth-thiolutin plates (for *tln-I*) or on minimal-thiolutin plates (for *tln-II*). In crosses involving thiolutin-sensitive *Hfr*s, the mating mixture was plated immediately on selection plates to score for prototrophic recombinants. The recombinants were purified by restreaking twice on the same type of selection plates and tested for thiolutin sensitivity by streaking on appropriate drug plates.

f) Transduction. Pl *vir a* obtained from N. Willetts was used throughout. Donor lysates with titres of 10^9 to 10^{10} PFU/ml were prepared by the confluent lysis technique. Transductions were performed as described by Miller (1972). Transductants were purified twice on appropriate selection plates before scoring for thiolutin resistance.

Results

1. Mapping of the tln-I Mutation

The thiolutin resistant mutant NSJ 74 (Table 1) was originally isolated in an *Hfr* strain (KL 16). It is resis-

Table 2. Conjugational mapping of *tln-I*

Cross	Selected markers	Number of recombinants tested	Percentage Cotransfer of Unselected markers				
			tln-I$^+$	*trp*$^+$	*purE*$^+$	*lac*$^+$	*leu*$^+$/*ara*$^+$
KL 208 × NSJ 76	*trp*$^+$	320	7.5	100	22	–	–
(HfrB7)	*purE*$^+$	145	56	–	100	–	–
KL 226 × NSJ 76	*purE*$^+$	166	45	–	100	–	–
(HfrC)	*lac*$^+$, *purE*$^+$	113	75	–	100	100	–
AB 259 × NSJ 76	*lac*$^+$	170	50	–	38	100	–
(HfrH)	*purE*$^+$	172	86	–	100	–	–
HfrB4 × NSJ 76	*ara*$^+$	125	0	–	–	–	100
HfrB8 × NSJ 76	*purE*$^+$	95	30	–	100	–	–

tant to thiolutin in minimal-broth-drug medium, but sensitive in minimal-drug medium (Sivasubramanian and Jayaraman 1976). At first we attempted to transfer the resistance marker to a multiply auxotrophic recipient (CSH 57b) by conjugation. We encountered several difficulties in directly selecting for thiolutin-resistant recombinants. While it was possible to transfer the resistance marker directly by conjugation the yield of thiolutin-resistant recombinants fluctuated widely from experiment to experiment. Their number also varied widely depending upon the medium used to select such recombinants. Several spurious 'recombinants' appeared after prolonged incubation, especially on complete broth-drug plates. These spurious colonies failed to grow when restreaked on the same type of plates. One probable reason for their appearance could be the inactivation of the drug by media components, as has been suggested earlier (Khachatourians and Tipper 1974b). These difficulties hampered quantitative assessment of recombination frequency. Therefore, we decided to map the thiolutin sensitive allele instead of the resistant one using a thiolutin resistant recipient which was constructed as follows:

a) Construction of NSJ 76 (CSH 57b tln-I). A multiply auxotrophic, thiolution sensitive recipient (CSH 57b) was chosen to transfer the *tln-I* mutation from the *Hfr* strain. This strain was mated with NSJ 74 (*Hfr tln-I*) for 90 min and grown in streptomycin containing broth overnight. Thiolutin-resistant recombinants were isolated from the segregated culture by spreading on minimal-broth-drug plates (see Materials and Methods, section (e). The spurious thiolutin resistant colonies were eliminated by restreaking two or three times on fresh drug plates. About 50 authentically thiolutin resistant colonies were picked up and checked for the retention of recipient markers. One recombinant which had retained all the recipient markers except *his* was chosen for further mapping. This strain (designated as NSJ 76) is resistant to the drug in minimal-broth-drug medium, but sensitive in minimal-drug medium.

b) Conjugational Mapping of tln-I. NSJ 76 was first mated with KL 208 (*HfrB7*). Prototrophic recombinants for all the markers were selected and purified twice on selective plates. The recombinants were then screened for thiolutin sensitivity on minimal-broth-drug plates. A good proportion of *purE*$^+$ recombinants were thiolutin sensitive indicating proximity of the *tln-I* locus to *purE* (Table 2). Mating was then carried out with KL 226 (*HfrC*) which transfers *purE* as an early marker. The data presented in Table 2 show that the closest linkage of thiolutin sensitivity was obtained with *lac*$^+$–*purE*$^+$ recombinants. Crosses with AB 259 (*HfrH*) also yielded the same pattern. These results suggested that the *tlnI* mutation could lie in the vicinity of *lac–purE*.

In order to map the position of *tlnI* precisely, conjugation experiments were carried out with a set of sibling *Hfr*s viz., *HfrB4* and *HfrB8*. These two strains transfer the chromosome clockwise and counter-clockwise respectively, each transferring *lac* as one of the last markers (see Fig. 1). None of the *ara*$^+$ recombinants in the *HfrB4* × NSJ 76 cross was thiolutin sensitive (Table 2) indicating the position of *tlnI* away from the origin of transfer of *HfrB4*, i.e., towards *purE*. It could lie between the origin of transfer of *HfrB8* and *purE* or beyond *purE* clockwise. It was found that only 30% of the *purE*$^+$ recombinants in the *HfrB8* × NSJ 76 cross acquired thiolutin sensitivity (Table 2). The low coinheritance of thiolutin sensitivity with *purE* in this cross suggested that the *tlnI* locus could lie between the origin of transfer of *HfrB8* and *purE* (9.0 to 11.5 min) since

Table 3. Map position of *tln-I*

Donor	Recipient	Selected Marker	Unselected Marker	Cotransduction Frequency (%)
NSJ 74	CSH 57b	*purE*$^+$	*tln-I*	1.2 (4/340)
NSJ 74	χ478	*proC*$^+$	*tln-I*	0.67 (2/288)
			purE$^+$	0 (0/288)
NSJ 74	AX 727	*dnaZ*$^+$(*ts*$^+$)	*tln-I*	46 (81/176)

it is known that markers lying close to the leading end of the transferred chromosome recombine poorly (Low 1965).

c) Transductional Mapping of tlnI. P1 transductions were then carried out to locate more precisely the map position of *tlnI*. For this purpose, recipient strains (CSH 57b, χ478, AX 727) having auxotrophic and temperature sensitive mutations in the *lac – purE* region were chosen. P1 *vir* propagated on *tln-I* (NSJ 74) donor was used to transduce various thiolutin sensitive recipients. As shown in Table 3, 1.2% of *purE*$^+$ and 0.67% of *proC*$^+$ transductants were thiolutin resistant. Since *tln-I* is cotransducible at low frequency with *purE* or *proC* but *purE* and *proC* themselves are not cotransducible (Table 3) *tln-I* must be located between *proC* and *purE*. Walker et al. (1977) have demonstrated that *dnaZ* is cotransducible with *purE* at a frequency of approximately 4.5% but not with *proC* (less than 0.53%). The lower co-transduction frequency of *tln-I – purE* (1.2%) than *dnaZ – purE* (4.5%) indicates the position of *tln-I* – to the left of *dnaZ*, i.e. between *proC* and *dnaZ*. To verify this, a *dnaZ ts* recipient (AX 727) was transduced to temperature insensitivitiy using NSJ 74 as donor, temperature insensitive transductants were isolated and checked for thiolutin resistance. The results show (Table 3) that 46% of the *dnaZ*$^+$ transductants were thiolutin resistant confirming the position of *tln-I* between *proC* and *dnaZ*. Using Wu's formula (Frequency of contransduction = $(1\text{-}d/L)^3$, where 'd' is the distance between markers and 'L' is the length of the transducing fragment, taken as 2 min) it could be calculated that *tln-I* lies 1.6 min clockwise from *proC* and 0.3 min and 1.5 min counter-clockwise from *dnaZ* and *purE* respectively i.e., at approximately 10.2 min on the linkage map. Figure 2 presents a summary of the transduction data. Attempts to transduce thiolutin resistance directly into recipient strains were unsuccessful for unknown reasons.

2. Mapping of the tln-II Mutation

The parental strain NSJ 75 (Table 1) was derived from NSJ 74 by mutagenesis (Sivasubramanian and

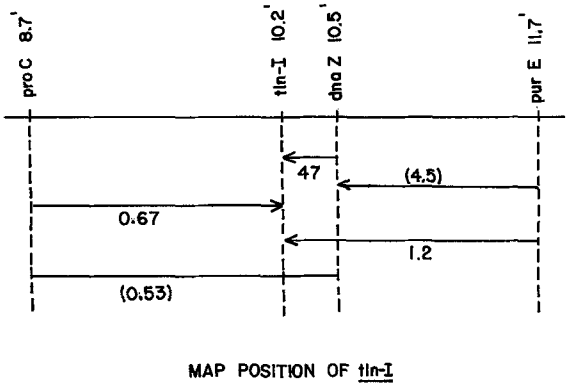

Fig. 2. Map position of *tln-I*. The figure represents the portion corresponding to 8.7–11.7 min of the recalibrated genetic map. The numbers above the arrows represent cotransduction frequencies and the arrowheads indicate the unselected markers. The numbers in parenthesis are from Walker et al. (1977)

Table 4. Linkage relationship between *tln-I* and *tln-II*

Donor	Recipient	Selected marker	Number of recombinants tested	Thiolutin resistance (%)	
				Minimal-broth-drug medium	Minimal-drug medium
KL 226 (HfrC)	NSJ 77	*purE*$^+$	153	99	52
		lac$^+$, *purE*$^+$	150	100	42

Jayaraman 1976) and is probably a double mutant. It is resistant to thiolutin in minimal-drug medium. The *tln-II* locus in this strain was also mapped using the same strategy as described above for *tln-I*.

a) Construction of NSJ 77 (CSH 57b tln-I, tln-II). NSJ 76 which is an F^- strain carrying *tln-I* was crossed with NSJ 75 and *trp*$^+$ recombinants were isolated. These were scored for thiolutin resistance on minimal-drug plates. A clone which was so resistant and which had retained all auxotrophic markers (except *his* and *trp*) was chosen for further mapping and designated as NSJ 77.

b) Linkage Relationship Between tln-I and tln-II Mutations. If *tln-II* lies very close to *tln-I* (within the *lac – purE* segment or very close on either side) replacement of the *lac-purE* segment of NSJ 77 by the wild-type alleles should confer thiolutin sensitivity on all media i.e., wild-type phenotype. To test this notion NSJ 77 was crossed with KL 226 (*HfrC*), *purE*$^+$ or *purE*$^+$-*lac*$^+$ recombinants were isolated and tested for thiolutin sensitivity on minimal-drug and minimal-broth-drug plates. The data presented in Table 4

shows that among such recombinants more than 99% were thiolutin resistant on broth supplemented plates while only 40%–50% were resistant on minimal plates.

The point we wish to emphasize here is that none of the $purE^+$ or $purE^+$-lac^+ recombinants acquired total thiolutin sensitivity. If *tln-I* and *tln-II* were closely linked we should have obtained such a class. This argues agains close linkage between *tln-I* and *tln-II*. Since *tln-I* maps in the *lac-purE* segment, a fraction of lac^+ or lac^+-$purE^+$ recombinants should be of the type $tlnI^+$-*tlnII*. The data presented in Table 4 shows that approximately 50%–60% of such recombinants lose the parental property, viz., resistance in minimal-drug medium. However all of the lac^+ or lac^+-$purE^+$ recombinants are still thiolutin resistant in broth-supplemented minimal medium. This shows that *tlnII* mutation is necessary, but not sufficient for resistance in minimal medium; the presence of both *tlnI* and *tlnII* mutations seems to confer resistance in minimal medium.

c) Construction of NSJ 78 (CSH 57b tln-I$^+$ tln-II.) Since the map position of *tln-I* is already established it was decided to separate *tln-I* and *tln-II* to facilitate mapping of the latter. NSJ 77 (CSH 57b *tln-I tln-II*) was crossed with KL 226 (*HfrC*) for 15 min to transfer the $tln\text{-}I^+$ allele, interrupted to prevent the transfer of distal markers and $purE^+$ recombinants were selected. These were checked for thiolutin sensitivity. It was found that some of the $purE^+$ recombinants were thiolutin-sensitive on minimal-drug plates but could grow on minimal-broth-drug plates. Moreover such recombinants grew at slightly lower concentrations of the drug showing the low level of resistance due to *tln-II*. One such clone was purified and designated as NSJ 78 (CSH 57b *tln-II*).

d) Conjugational Mapping of tln-II. NSJ 78 was crossed with several *Hfr*s, recombinants for various markers selected, segregated and checked for thiolutin sensitivity on minimal-broth-drug plates. In crosses with KL 226 (*HfrC*) and *Hfr* KL 14 a good proportion (65% and 35% respectively) of the xyl^+ recombinants showed thiolutin sensitivity suggesting the position of *tln-II* near *xyl*, probably clockwise from *xyl* (Table 5). In order to map the position of *tln-II* precisely, *Hfrs* having origins of transfer near *xyl* –*ilv* – *metE* were chosen. Two such strains (KL 25 and KL 209) transfer *ilv* counter-clockwise and clockwise respectively. NSJ 78 was crossed with these donors and met^+ recombinants were isolated. As shown in Table 5, approximately 30% of the met^+ recombinants in these crossed showed thiolutin sensitivity, whereas in the cross with *Hfr* KL 228, which transfers

Table 5. Conjugational mapping of *tln-II*

Cross	Selected Marker	Number of recombinants tested	Percentage of co-transfer of $tln\text{-}II^+$
KL 226 × NSJ 78	ara^+	100	0
(HfrC)	$metA^+$	99	0
	xyl^+	87	65
KL 14 × NSJ 78	$argG^+$	100	0
	xyl^+	103	36
	xyl^+, ilv^+	104	99
KL 25 × NSJ 78	$metA^+$	65	30
KL 209 × NSJ 78	$metA^+$	94	32
KL 228 × NSJ 78	xyl^+	90	1
	$argG^+$	75	3

Table 6. Map position of *tln-II*

Donor	Recipient	Selected marker	Unselected marker	Cotransduction frequency (%)
NSJ 75	AT 2538	$pyrE^+$	*tln-II*	0 (0/99)
NSJ 75	AB 3505	$ilvD^+$	*tln-II*	66 (99/150)
			$metE^+$	17 (26/150)
		$metE^+$	*tln-II*	90 (116/130)
			$ilvD^+$	96 (125/130)

ilv as the last known marker, only 1% to 3% of the xyl^+ or $argG^+$ recombinants showed thiolutin sensitivity. This indicated the position of *tln-II* closer to *ilv* rather than *xyl*.

In all these crosses the yield of ilv^+ recombinants was very poor and even the few which were obtained grew poorly. Therefore the coinheritance of *tlnII* with *ilv* could not be determined. The same difficulty in recovering ilv^+ recombinants was encountered by Chakraborti and Maitra (1974) in their studies. We have no explanation for this phenomenon.

e) Transductional Mapping of tln-II. A P1 *vir* lysate propagated on NSJ 75 was used to transduce thiolutin sensitive recipients (AT 2538, AB 3505) having mutations around the *ilv* region (*pyrE*, *ilvD* and *metE*). Prototrophic transductants were selected, segregated and checked for the acquisition of thiolutin resistance. As shown in Table 6, none of the $pyrE^+$ transductants showed thiolutin resistance which indicates the position of *tlnII* closer to *ilvD*. When $ilvD^+$ transductants were selected, 66% of them were thiolutin resistant while only 17% were $metE^+$. This suggested that the *tlnII* lies in between *ilvD* and *metE*, closer to the former. However, when $metE^+$ transductants were selected in the same cross, a majority of them (90%–

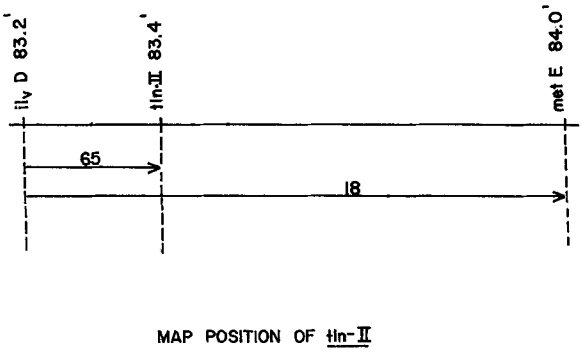

Fig. 3. Map position of *tln-II*. The figure represents the portion corresponding to 83.2–84 min of the recalibrated genetic map. The numbers above the arrows represent contransduction frequencies and the arrowheads indicate the unselected markers

96%) were thiolutin resistant and also *ilvD*$^+$. We do not know the reasons for this anomolous result. It may be pointed out that the *metE – ilvD* segment is an error prone region, as revealed by several studies (Bachmann et al. 1976). The frequency of occurrence of the unselected marker depends upon which of the pair is the selected marker and can vary several fold. Therefore the high cotransduction of *tlnII* with *metE* has to be interpreted with caution. It has been shown that the gene for RNA chain termination factor *rho* is cotransducible with *ilvD* by 65% (Das et al. 1976) to 82% (Inoko and Imai 1976; Morse and Primakoff 1970). The cotransduction frequency of *tlnII* with *ilvD* indicates that *tlnII* lies very close to the *rho* locus. Figure 3 presents a summary of the transduction data.

Discussion

There are several ways by which a bacterial cell could develop resistance to a metabolic inhibitor. Mutations which reduce or abolish transport of the agent, inhibit its conversion to active form in vivo, induce inactivating enzymes, reduce the affinity of the target molecules etc., could, in principle, confer resistance to the agent. As far as thiolutin resistance is concerned all the possibilities except the last one could be ruled out. The studies of Tipper (1973) suggested that thiolutin is probably not converted into an active form in vivo. Our earlier observations (Sivasubramanian and Jayaraman 1976: Sivasubramanian 1979) have revealed that, although the thiolutin resistant mutants grow in the presence of the drug, processes such as induction of β-galactosidase, development of phages T4, T7 and λ are still inhibited totally or partially. Thus resistance to thiolutin does not seem to involve transport, activation or inactivation. We are, therefore, left with the last of the above mentioned possibilities, viz., reduction in the affinity of the target molecules. As has been pointed out in the Introduction, the inhibitory action of thiolution on transcription in vivo is not mediated by its action on the template or RNA polymerase. It is, therefore, logical to conclude that some accessory factor(s) involved in transcription in vivo might be the targets of thiolutin and mutations in the loci coding for them result in thiolutin resistance. The properties of these mutants (Sivasubramanian and Jayaraman 1976; Sivasubramanian 1979) lend some support to this view. While the relative incorporation of ^{3}H-uridine in the mutants in the presence and absence of thiolutin was higher than the thiolutin sensitive parent strain, the incorporation itself was quite low compared to the parent even in the absence of the drug (Sivasubramanian and Jayaraman 1976). Moreover the mutants grow with a very long generation time relative to the parent, particularly in minimal media (Sivasubramanian and Jayaraman 1976; Sivasubramanian 1979). These observations suggest that the mutations are such that, while they confer resistance to thiolutin, they also result in a decrease in the overall efficiency of transcription.

The possible existence of at least two loci involved in thiolutin sensitivity/resistance was evident from our earlier work (Sivasubramanian and Jayaraman 1976). Mutants harbouring the *tln-I* mutation exhibited resistance only in rich or partially rich media but were sensitive in minimal media. Mutants resistant to thiolutin in minimal media were obtained from these by further mutagenesis. These were then tentatively assumed to be double mutants harbouring *tln-I* and *tln-II* mutations. In the present study we have confirmed that they are indeed double mutants. The *tln-I* and *tln-II* loci have been mapped around 10.2 min and 88.5 min respectively on the genetic map. The interesting observation made here is that the presence of both *tln-I* and *tln-II* mutations are necessary for resistance in minimal media. Either one of them in isolation renders the cell 'conditionally auxotrophic'. It is conceivable that these two loci code for accessory transcription factors involved in different sets of transcriptional events.

Procaryotic transcription involves the participation of several accessory transcription factors (see review by Doi 1977). Travers (1976) has suggested that RNA polymerase of *E. coli,* is an allosteric enzyme whose activity and specificity could be controlled by a variety of cellular metabolites such as ppGpp, EF-TuTs and f-met tRNA. These effectors could either alter the specificity of RNA polymerase or change the distribution between different specificity forms. Genetic and physiological evidences are available for these changes in RNA polymerase specificity. RNA polymerase isolated from a mutant (*ts 103*) originally

obtained by Jacobson and Gillespie (1970) behaves as though the equilibrium between the low and high affinity forms for ribosomal RNA transcription is shifted in favour of the high affinity isomer. The *ts 103* mutation maps at a locus different from those defining the subunits of RNA polymerase (Travers 1976). Similarly the *alt 1* mutation is believed to change the distribution of functionally distinct RNA polymerase isomers (Travers et al. 1978). In T4-infected cells a phage-coded polypeptide of molecular weight 15,000 has been shown to be necessary for the reduction in the affinity of RNA polymerase to rRNA promoters and phage mutants defective in this function are known (Sirotkin et al. 1977). In addition Ratner (1974) has noted that several proteins bind to *E. coli* RNA polymerase immobilized on agarose. The thiolutin sensitive factors also could be involved, directly, or indirectly through effectors, in determining the specificity and/or the distribution of different specificity isomers of RNA polymerase. The insensitivity of RNA polymerase to thiolutin in vitro and the map position of the mutations conferring thiolutin resistance show that RNA polymerase per se is not the target of the drug. Isolation of the thiolutin sensitive factors and elucidation of their mode of action should throw more light on transcription and its control in *E. coli.*

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