ON SOME GENETIC ASPECTS OF PHAGE λ RESISTANCE IN *E. COLI* K12

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ABSTRACT

Most mutations rendering *E. coli* K12 resistant to phage λ, map in two genetic regions *mal*A and *mal*B.——The *malB* region contains a gene *lam*B specifically involved in the λ receptor synthesis. Twenty-one independent *lam*B mutations studied by complementation belonged to a single cistron. This makes it very likely that *lam*B is monocistronic. Among the *lam*B mutants some are still sensitive to a host range mutant of phage λ. Mutations mapping in a proximal gene essential for maltose metabolism inactivate gene *lam*B by polarity confirming that both genes are part of the same operon. Because cases of intracistronic complementation have been found, the active *lam*B product may be an oligomeric protein.——Previously all λ resistant mutations in the *mal*A region have been shown to map in the *mal*T cistron. *mal*T is believed to be a positive regulatory gene necessary for the induction of the "maltose operons" in the *mal*A region and in the *mal*B region of the *E. coli* K12 genetic map. No *trans* dominant *mal*T mutation have been found. Therefore if they exist, they occur at a frequency of less than 10^{-8}, or strongly reduce the growth rate of the mutants.

WHEN λ-resistant (λr) mutants are selected in cultures of *E. coli* K12 about 80% of them are unable to utilize maltose as a carbon source (Mal^-) (LEDERBERG 1955). Most of these λrMal^- mutations map in the monocistronic *mal*T gene of the *mal*A region (Figure 1) (SCHWARTZ 1967; HATFIELD, HOFNUNG and SCHWARTZ 1969; HOFNUNG, HATFIELD and SCHWARTZ 1971), while some rare λrMal^- mutations map in the *mal*B region (SCHWARTZ 1967a). Of the 20% other λr mutants which can utilize maltose (Mal^+), at least some have been shown to be tightly linked with the *mal*B region (SCHWARTZ 1967b). A model has been proposed (SCHWARTZ 1967b), which accounts for the preceeding facts and which explains why in some strains of *E. coli* K12, maltose induces the synthesis of the receptors for phage λ and the synthesis of the enzymes necessary for the metabolism of maltose. In this model, the *mal*T gene product acts as a positive regulator for the induction by maltose of at least two operons, one of which located in the *mal*A region has been characterized (HATFIELD *et al.* 1969). It contains the structural genes for amylomaltase (*mal*Q) and maltodextrin phosphorylase (*mal*P), two enzymes of the maltose metabolism. Another operon located in the *mal*B region has not been characterized so thoroughly. It would be

made of a gene involved in maltose permeation (called the *mal*B gene. or *mal*B) and a gene, *lamB* (previously called λ *rec*B), involved in the synthesis of the cell surface receptors for phage λ (SCHWARTZ 1967b)*.

Here, we report a more detailed analysis of the different type of λr mutations and especially those located in the *mal*B region. We have selected mutants resistant to phage λ in strains diploid for *mal*T, or both *mal*T and the *mal*B region. We have found that in the *mal*B region, gene *lam*B controlling the synthesis of the phage λ receptors is made of one cistron and that the *lam*B gene product is most probably an oligomeric protein since some intracistronic complementation has been detected. *lam*B and the gene involved in maltose permeation belong indeed to the same operon (SCHWARTZ 1967b) since the existence of polar mutations has been confirmed. Strain CR63 is resistant to λ and sensitive to host range mutants of λ (λrλhs) (APPLEYARD *et al.* 1956). The corresponding mutation has been mapped in *lamB* and more mutations of this type have been isolated.

MATERIALS AND METHODS

Abbreviations and genetic markers: Genetic markers are as recommended by TAYLOR (1970) except for *str*^{S} and *str*^{R} which mean sensitive and resistant to streptomycin respectively. The phenotypic abbreviations are as follows: λr and λs mean resistant and sensitive to phage λ virulent (λv) respectively. λhr and λhs mean resistant and sensitive to phage λh virulent (λhv) respectively. Mal+ and Mal− mean able and unable to use maltose as a carbon source.

Merodiploids are represented as follows: on the upper line are the alleles of the episome (F′ means F′ factor, φ80 means phage φ80) and on the lower line are the alleles of the bacterial chromosome.

Bacterial strains, media, bacteriological techniques: The strains used are listed in Table 1. The location of the genetic markers on the linkage map of *E. coli* K12 is shown in Figure 1. Media, techniques for transduction and bacterial mating were as described elsewhere (HOFNUNG and SCHWARTZ 1971).

*rec*A recombinants were scored by their sensitivity to ultraviolet light. Meso-α-ε- diaminopilelic acid (DAP) was used at a concentration of 0.01% and streptomycin at a concentration of 50 μg/ml. The *asd* mutation leads to a requirement for DAP even in ML (complete) medium. This property is used to select against the loss of an episome carrying the *asd*+ allele.

Episomes: F′ KLF41 and F′ KLF12 carry portions of the bacterial chromosome located approximately between minutes 60 and 67 and between minutes 77 and 84 respectively. F′ KLF41 carries the alleles *arg*G+, *str*^{S}, *aro*B+, *mal*A+ and *asd*+; F′ KLF12 carries *arg*H+, *met*A+, *mal*B+ and *pyr*B+ (personal communication from BROOKS LOW, and unpublished results).

Transducing phage φ80 $dmal_2$ has been already described (HOFNUNG, HATFIELD and SCHWARTZ 1971) and is a gift of J. BECKWITH and D. SCHWARTZ. λCI_{857}t68h80 $dmal_2$ is a recombinant between λCI_{857}t68h80 and φ80 $dmal_2$ made by M. SCHWARTZ. Both phages carry genes *mal*T, *mal*P and *mal*Q. They are defective but integrate without helper at the *att*80 locus of the bacterial chromosome.

F′ episomes were always used in *rec*A strains: no recombination between episome and chromosome was ever observed by us in these strains.

Phages and phage stocks: The P1 strain used is a partially virulent mutant of P1kc from DR. S. BRENNER. λv, a virulent mutant of λ+ (JACOB and WOLLMAN 1964), was used to select λr mutants. λhv, a host range mutant of λv, was isolated as a spontaneous mutant by plating λv on strain CR63 (APPLEYARD, MCGREGOR and BAIRD 1956). The same isolate of λhv was used

* It is now known (manuscript in preparation) that in the *mal*B region there is more than one gene which would be involved in maltose permeation. However, in the present paper, we call *mal*B the cluster of genes of the *mal*B region in which non polar point mutations result in a λs*Mal*− phenotype. *lam*B point mutants are λr*Mal*+.

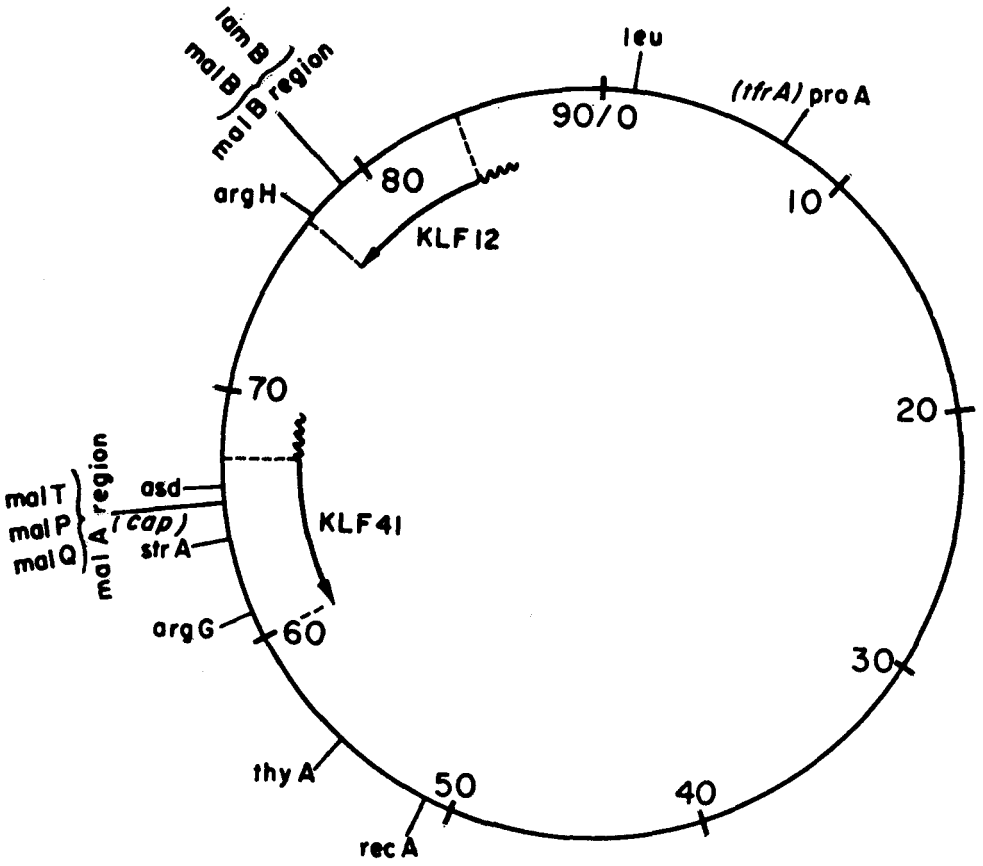

FIGURE 1.—Map location of the genetic markers used in this study (nomenclature as recommended by TAYLOR 1970).

throughout this study. Its efficiency of plating is about the same on C600 and CR63; it has not been further characterized.

Stocks of λv (10^{11} pfu/ml) were kept at 4°C in ML medium, 10 mM magnesium sulfate and 5% chloroform. The titer of λv did not change appreciably for at least six months, while during the same time the titer of λhv mutants in the stock decreased by a factor of about one hundred. When used, the λ stocks contained about 10^3 pfu of λhv per ml, the presence of which was of no importance for the experiments since λrλhs mutants were isolated with these very stocks.

Plate test for λ sensitivity: Bacteria to be tested for λ sensitivity were cross-streaked with a λv suspension of only 10^7 pfu/ml (to avoid the presence of λhv) on either EMB mal plates or EM mal when the strain was Mal+, or on minimal gal or lac medium when the strain was Mal−. Minimal media were supplemented with the necessary requirements and were used each time segregation of an episome was to be avoided.

A more sensitive test consisted in spotting dilutions of a λv stock on a lawn of 10^8 to 10^9 bacteria on a plate containing one of the media above. All plates were incubated overnight and then scored for lysis.

Selection of mutants resistant to phage λ: Bacteria were grown in 5 ml of ML medium or in a medium selectively retaining the episomal genes of the F′ merodiploids. To induce the synthesis of the phage λ receptors the cultures were made 0.4% with respect to maltose when cell density reached 5×10^8 cells/ml. They were then grown for 30 more minutes. The bacteria

TABLE 1

Bacterial strains

Name	Mating type	Chromosome	Episome	Observation
$HfrG_6$	Hfr	*his* str^s		From MATNEY
pop1000	Hfr	*his* $malQ_7$ str^s		EMS-induced *malQ* mutant of $HfrG_6$ (HATFIELD *et al.* 1969)
pop1001	Hfr	*his* str^s *malT*+ $malQ_7$	$\phi 80$ *malT*+ *malQ*+	Lysogenisation of pop1000 with $\phi 80$ $dmal_2$ (HOFNUNG *et al.* 1971)
pop1002	Hfr	*his* str^s *malT*+ $malQ_7$	λ*malT*+ *malQ*+	Lysogenisation of pop1000 with λCI_{857} t68h80 $dmal_2$ (HOFNUNG *et al.* 1971)
HfrKL166	Hfr	*recA* str^s		(See HOFNUNG *et al.* 1971)
pop338	F−	*argG metA asd thy his* str^r		.....
pop342	F−	*recA argG metA asd* str^r		Recombinant between HfrKL166 and pop338
pop421	F−	*recA argH malB*(MBΔ101) str^r		The *malB* mutation (MBΔ101 inactivates all known genes in *malB* (SCHWARTZ 1967b)
pop422	F−	*recA argG pro malB*(MBΔ101) str^r		NTG induced Pro− mutant of pop421
pop424	F−	*recA argH thyA malB*(MBΔ101) str^r		Spontaneous Thy− mutant of pop421
PA505-MBΔ101	F−	*argH metA malB*(MBΔ101) str^r		See SCHWARTZ 1967b)
pop366	F−	*recA argG metA* $malQ_6$ str^r		.....
CR63	F−			See APPLEYARD *et al.* (1956) resistant to λ sensitive to λh
C600	F−	*thr leu* str^s		.....
$\phi 3$–3876–2	F−	*leu malB* (MBΔ21)		From RICHTER (1961)
JC1553 (KLF41)	F′	*recA leu metB his argG* str^r *malA asd*	F′ *argG*+ str^s *malA*+ *asd*+	From BROOKS LOW
pop480	F′	*recA metA argG* str^R *malA*+ *asd*	F′ *argG*+ str^s *malA*+ *asd*+	Recombinant between pop342 and JC1553 (KLF41)
pop478	F′	*recA pro* str^R *argH*+ *malB* (MBΔ101)	F′ *argH*+ *malB*+	Introduction of episome KLF12 (from BROOKS Low) into pop422

pop479	F′	*rec*A *arg*G *str*^{R} *met*A *mal*B+ *mal*T+ *mal*Q_6	F′ *met*A+ *mal*B+ φ80 *mal*T+ *mal*Q+	Lysogenisation of pop366 with φ80 d*mal*$_2$ and introduction of KLF12

Only the relevant genotype has been indicated. Most strains require B1. When no origin is indicated, the strain is from the laboratory collection. Mutation MBΔ101 is a deletion which inactivates gene *lam*B and at least one gene essential for the metabolism of maltose in *mal*B (SCHWARTZ 1967a). MBΔ21 is our name for the *mal*$_5^-$ mutation of Richter (RICHTER 1961).

were then centrifuged, resuspended in 0.5 ml of 10 mM magnesium sulfate, infected with λv at a multiplicity of at least 10, incubated at 37°C for 30 min and diluted with 5ml of ML medium. They were aerated at 37°C until lysis (2 to 3 hr), centrifuged, resuspended in 0.2 ml of 10 mM magnesium sulfate with 10^9 λv, and plated on the appropriate medium: EMB maltose, TTZ maltose or the adequate synthetic medium when the loss of an episome was to be avoided. When the strains had a thermoinducible prophage, the cultures were grown at 30°C instead of 37°C. *Mucoid clones surviving the infection with λv were not studied.* (Their frequency in the population was less than 10^{-7} in all cases and less than 10^{-10} in the selection with the strain diploid for both the *malA* and the *mal*B region. In this last case plating was done on EM maltose with the required supplements).

Episome curing: (a) F′KLF41——Strain pop480 and its λr derivatives, merodiploids of structure $\frac{\text{F′ } arg\text{G+ } str^S \text{ } mal\text{A+ } asd\text{+}}{arg\text{G } str^R \text{ } mal\text{A+ } asd}$ were grown in ML medium with DAP for about 16 generations and plated on EMB maltose plates containing DAP and streptomycin. Because *str*^{S} is dominant over *str*^{R}, most of the survivors had lost F′KLF41. Occasionally, some colonies still carried part of F′KLF41, deleted for at least the *str*+ allele as shown by subsequent genetic analysis. (b) *F′KLF12*——Pop 478 and its λr derivatives, merodiploids of structure $\frac{F′ \text{ } met\text{A+ } mal\text{B+}}{met\text{A } mal\text{B}}$, were grown in ML medium for about 10 generations and plated on EMB maltose plates. The white colonies had lost F′KLF12 as shown by genetic analysis.

Mutagenesis:—with ethyl methane sulfonate (EMS): 5 ml of a growing bacterial culture at a density of about 5×10^8 cells/ml were centrifuged and the bacteria resuspended in 2.5 ml of M63 medium together with up to 0.1 ml EMS. They were incubated and aerated for one hour at 37°C. The mutagen was removed by repeated centrifugation and washing with M63 medium. The bacteria were then resuspended in 5 ml of the appropriate medium with one ml of ML medium and grown for 10 hr at 37°C to allow the expression of the recessive mutations. With *rec*A strains only up to 0.01 ml EMS was used.

—with N-methyl-N′-nitro-N-nitrosoguanidine (NTG): Revertants of the λrMal$^-$ mutants were selected with a crystal of NTG on a plate seeded with 5×10^9 cells.

Assay of the amylomaltase: Amylomaltase was assayed on plates as already described (SCHWARTZ 1967b; HOFNUNG and SCHWARTZ 1971).

RESULTS

Mutations of λ resistance mapping outside mal*T:* Selection of λr mutants was done in merogenotes which were diploid for at least the *mal*T gene. F′KLF41 carries a large fraction of the bacterial chromosome covering the *malA* region, while φ80 *dmal*$_2$ carries only a very small portion of bacterial genes in excess of the *malA* genes (see MATERIALS AND METHODS).

(a) *Isolation of λr mutants from two strains, both diploid for* mal*T, carrying either KLF41 or φ80* dmal$_2$*:* With pop480, twenty spontaneous independent λrMal$^+$ (LMP 11 to 30) and twelve spontaneous independent λrMal$^-$ (LMM 2,4,5,6,9,211,212,213,311,321,331,341) mutants were isolated. The frequency of these two types of mutants in the population was about 10^{-6} and 5×10^{-8} respectively. Three other clones of pop480 were mutagenized with EMS, and infected with λv. Three λrMal$^+$ (LMP 36,37,38) and three λrMal$^-$ (LMM 361, 371, 381) independent mutants were isolated.

The thirty eight mutants were cured of their F′KLF41, and F′KLF12 was introduced in every one of them. All the merodiploids thus constructed were phenotypically Mal$^+$ and λs. Therefore the thirty-eight Mal$^+$ λr and Mal$^-$ λr mutations map in the region covered by F′KLF12 and are recessive to the wild-type alleles. As a control, F′ KLF41 was reintroduced in each mutant: all the corresponding merodiploids remained λr.

With pop1001, fifty spontaneous λrMal$^-$ and fifty spontaneous λrMal$^+$ mutants were selected from four clones. Two more clones of pop1001 were mutagenized with EMS and fifty λrMal$^-$ and fifty λrMal$^+$ mutants were isolated.

Among the one hundred λrMal$^-$ mutants isolated in pop1001, ninety eight were constitutive for the synthesis of amylomaltase (notation A^c) as detected by the plate test for the enzyme. Five of these ninety eight mutations were tested and found to map in the *mal*B region since they did not give any Mal$^+$ recombinants with deletion MBΔ101. This deletion is known to inactivate all genes of the *mal*B region (including gene *lam*B) (SCHWARTZ 1967b and manuscript in preparation). Thirty five mutants of HfrG$_6$ selected for the double phenotype "λr and constitutive synthesis of Amylomaltase" were also Mal$^-$ (SCHWARTZ 1967b; HATFIELD private communication) and the genetic defect was in the *mal*B region. This, together with the mapping of the five mutations above suggests strongly that all the mutations resulting in the triple phenotype λrMal$^-$ A^c in HfrG$_6$ or its derivatives belong to the same class of mutations and map in the *mal*B region.

The two remaining mutants (LMM1E2 and LMM3E1) among the one hundred λrMal$^-$ mutants had a non-inducible basal level of amylomaltase and reverted to λsMal$^+$. Both mutants had a recessive mutation on the phage and on the chromosome because lysogenisation of the mutants with λCI$_{857}$t68h80 *dmal*$_2$ converted them to the phenotype λsMal$^+$. Probably these mutations lie in *mal*T. No *mal*T transdominant mutant and no λr mutant resulting from a mutation outside *mal*T and the region covered by F′KLF12 were isolated in these experiments. Their frequency in the bacterial population, if they exist, is therefore at least two orders of magnitude less than that of λrMal$^-$ mutants in the *mal*B region (i.e. less than 10^{-8}).

(b) *Isolation of λ resistant mutants in strains diploid for* mal*T and the* mal*B region:* The results above have shown that all the λ resistant mutations isolated by us and occuring outside the region covered by F′KLF41, mapped in the region covered by F′KLF12. We expect that the frequency of λr mutations in other regions of the chromosome should be at least two orders of magnitude less. This

expectation was tested with eight clones of pop479 diploid for *mal*T$^+$ and for the region covered by F'KLF12 which were mutagenized with EMS, infected with λv, grown and plated on EM maltose plates supplemented with arginine. One hundred and nine Mal$^+$ survivors were isolated. Their frequency in the population was about 10^{-8}. They were all λs although four of them to a much lesser degree when tested with different concentrations of λv.

In a strain diploid for *mal*T and the region covered by the KLF12 episome, it was thus impossible to find λrMal$^+$ mutants at a frequency higher than 10^{-10}. This low frequency indicates that except for genes located in the region covered by F'KLF12 and genes taking part in maltose metabolism, or in a process whose inactivation by mutation strongly impair the capacity of the cell to grow, there are no other genes involved in the synthesis of the phage λ receptors.

λrMal$^+$ mutants:

(a) *Isolation of λrMal$^+$ mutants in the* mal*B region:* Fifteen independent spontaneous λrMal$^+$ mutants (LMP 411 to 426) were isolated from pop478 which carries deletion MB101 on its chromosome (see above), and a wild-type *mal*B region only on the F'KLF12 episome.

All fifteen λrMal$^+$ mutations were carried by the episome. This was shown by curing the fifteen mutants of their F'KLF12 and by reintroducing the wild-type episome. All the strains thus constructed were again λs.

The λrMal$^+$ mutations are recessive to wild type. When each of the fifteen F'KLF12 carrying the λrMal$^+$ mutations were introduced into λs strain pop342 they did not make this strain λr.

The λrMal$^+$ mutations map in the region inactivated by deletion MBΔ101 (i.e. the *mal*B region). This results from the fact that when each of the episomes from the fifteen mutants were introduced into strain pop424 carrying deletion MBΔ101, the merogenotes thus constructed were λrMal$^+$.

(b) *The λrMal$^+$ mutations carried by F'KLF12 are all in gene* lam*B:* Ten of the fifteen λrMal$^+$ mutations above were separately introduced into eleven of the λrMal$^+$ mutants of pop342 (see § Ia, Table 2) by cross.

One hundred and seven of the 110 merodiploids were resistant to phage λ indicating that all 21 λrMal$^+$ mutations described above were located in gene *lam*B and belong to a single cistron. Three of the 110 merodiploids were λs and should represent cases of intracistronic complementation to λ sensitivity.

As stated above, when the wild-type KLF12 episome was introduced into each of the eleven λrMal$^+$ mutants of pop342, or when λrMal$^+$ derivatives of F'KLF12 were introduced into the λs strain pop343, the corresponding merodiploids were always λs. *lam*B$^+$ is therefore dominant over *lam*B whether on the episome or on the chromosome.

(c) *Among the λrMal$^+$ mutations in* lam*B, some are λhs:* Out of the twenty one *lam*B mutants isolated in pop342 and pop478, three (LMP 16, 18, 22) were like strain CR63 i.e. sensitive to λh and resistant to λ, suggesting that the mutation of CR63 maps in *lam*B. To ascertain this, a λr λhr Mal$^-$ *mal*B mutant (ϕ-3876-2) was transduced with a P1 stock grown on strain CR63. The twenty one Mal$^+$ transductants analyzed were λr λhs like CR63.

TABLE 2

Tests of complementation between different λr mutations

Mutation number	LMP 11, 12, 13, 14, 15, 17, 21, 24	LMP 18	LMP 16	LMP 22	LMM 212, 321, 331, 341, 361, 371	Wild type
LMP 411	—	— (+)	+	+	—	+
LMP 412	—	— (+)	— (+)	+	—	+
LMP 413, 414 415, 416	—	—	—	—	—	+
419, 421 422, 424		(+)	(+)	(+)		
Wild type	+	+	+	+	+	+

Top: λr mutations carried on the chromosome of pop342. Left: λr mutations carried on the KLF12 episome of pop478. Mutations of phenotype λr λhs are underlined. LMP = mutation resulting in a λrMal+ phenotype. LMM = mutation resulting in a λrMal- phenotype. The table indicates "+" if there is complementation, "—" if there is no complementation between λr mutations. Merodiploids were also tested for sensitivity to λhv : when the result differed from the λv test, it is indicated in brackets.

λrMal- mutations in the mal*B region:* A number of λrMal- mutations of the *mal*B region were isolated from strains diploid for *mal*T. They were always found to be recessive (diploid phenotype : λs Mal+) to wild-type F′ *mal*B when in merodiploids. Moreover, when twelve episomes carrying twelve different *lam*B mutations (λr Mal+) described in the preceeding paragraph were separately introduced into fifteen independent λr Mal- mutants isolated from strain pop480 (Table 2) the merodiploids formed were λr and Mal+. Therefore these λrMal- mutations have a purely *cis*-dominant effect for the resistance to phage λ and for the maltose phenotype. A large proportion of those λrMal- mutants (14 out of 15 in the case of strain pop480) revert at a high rate to wild-type phenotype; they are not deletions or double mutants. (In other strains, the proportion of non reverting λrMal- was higher. Some of them were shown to be deletions). The *cis*-dominant properties of the reversible λrMal- mutations and mapping data based on a series of deletion mutants (SCHWARTZ 1967b and manuscript in preparation), demonstrate that they are polar mutations located in a gene specifically involved in maltose permeation. This gene and *lam*B are therefore in the same operon. None of the reversible λrMal- mutations in *mal*B was suppressible by su_{III} or su_{IV} (GAREN 1968). They are presumably either frameshifts (MALAMY 1966) or insertions (JORDAN, SAEDLER and STARLINGER 1968; SHAPIRO 1969) exerting a polar effect on the expression of gene *lam*B.

The enzymes specific of maltose metabolism have been assayed in the λrMal- mutants of the *mal*B region. As expected all the mutants lacked maltose permease activity.

All the λrMal- mutants isolated in pop480 had a non inducible *mal*P–*mal*Q operon, while those isolated in pop1001, a derivative of $HfrG_6$, expressed this operon constitutively. It has already been reported that some *lam*B mutations could give a non-inducible or a constitutive *mal*P *mal*Q expression depending on the strain in which they are located, (SCHWARTZ 1967b).

DISCUSSION

The great majority of the λr mutations in *E. coli* K12 map in the *mal*A region or in the *mal*B region. On the average 80% of the λr mutants have a Mal⁻ phenotype, while the remaining 20% have an apparently normal maltose metabolism (LEDERBERG 1955; SCHWARTZ 1967b). All the λrMal⁺ mutations tested here map in the *mal*B region. Most of the λrMal⁻ mutations map in gene *mal*T (in *mal*A), the positive regulator gene of the maltose system. (HATFIELD *et al.* 1969; HOFNUNG *et al.* 1971). However, some of the λrMal⁻ mutations (about 1% of them) map in the *mal*B regions. λr mutations have been described which do not map in the *mal*A or in the *mal*B regions. For example, mutations in the *cap* gene (called also *crp* gene), a gene involved in catabolite repression, mapping between *malA* and *str*, make the cell resistant to phage λ (BECKWITH, personal communication; DE CROMBRUGGHE *et al.* 1971). Also, CURTISS has described a mutant resistant to λ as well as to other phages whose mutations map in the *pro*A region of the chromosome (CURTISS 1965). Why these λr mutations have escaped our selections is not clear. One of the reasons could well be that growth of these mutants was impaired so that their frequency was greatly reduced in the bacterial population. This was checked with one *cap* mutant: in ML medium it grew at a much lower rate than λr mutants in the *mal*A or in the *mal*B region.

The twenty-one λrMal⁺ mutations tested by complementation belong to a single cistron which is part of the *mal*B region. Therefore gene *lam*B defined as the locus for λr Mal⁺ mutations in the *mal*B region, is likely to consist of a single cistron. Intracistonic complementation occurs between some *lam*B mutations, suggesting that the active *lam*B product is an oligomeric protein. A priori, *lam*B could code either for an enzyme involved in the biosynthesis of the receptors or for a structural component of the receptors. The *lam*B mutants which are still λhs, like strains CR63, could owe their phenotype either to a decrease in the quantity or in a modification of the specificity of the *lam*B product. This point is being investigated.

*lam*B is part of an operon which also contains a gene involved in the metabolism of maltose. This conclusion can be drawn from the fact that λrMal⁻ mutations of *mal*B which map in a gene involved only in maltose metabolism and not in λ receptor synthesis (SCHWARTZ 1967b) inactivate gene *lam*B located in *cis* (but not in *trans*) position.

The results obtained here agree with the current model describing the regulation of the maltose system. According to this model, all the λr mutations we have described owe their phenotype to the inactivation of the same cistron, *lam*B. This inactivation is either direct (λrMal⁺ mutations located in the *lam*B cistron itself) or indirect (polar λrMal⁻ mutations in the *mal*B region or λ*rmal*⁻ mutations in *mal*T, the positive regulator gene of the system).

The model can account also for the old observation (WEISSBACH and JACOB 1962; HOWES 1965) than glucose represses the synthesis of λ receptors and the more recent result that mutants of the CAP protein, which are under permanent catabolite repression, are resistant to phage λ. (BECKWITH, personal communication; DE CROMBRUGGHE *et al.*, 1971). The CAP (or CRP) protein is acting as

a positive regulator activated by cyclic AMP. It is required for the expression of many of the genes encoding catabolite enzymes. The CAP protein might act directly to promote expression of the *mal*P *mal*Q operon in the *mal*A region (the *mal*P and *mal*Q genes are subject to catabolite repression) and of the *lam*B containing operon in the *mal*B region. Alternatively, the CAP protein might be involved in the expression of the *mal*T gene and promote indirectly the expression of the operons in the *mal*A and *mal*B region.

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