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Expression of *malT*, the regulator gene of the maltose regulon in *Escherichia coli*, is limited both at transcription and translation

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Six mutations, which lead to an increase in *malT* expression, were mapped by sequencing techniques. All of them had one or other of two base changes. Determination of the transcription start point by reverse transcriptase mapping localised the two base changes with respect to the elements that control *malT* expression. One of the base changes (*malTp1*) is located in the Pribnow box of the promoter, and presumably results in an increase in the rate of transcription initiation. The other (*malTp7*) is located in the Shine and Dalgarno sequence, which precedes the *malT* cistron. It probably created a more favourable ribosome binding site on *malT* mRNA. A correlate of these observations is that the promoter and the ribosome binding site are both inefficient in a wild-type *malT* gene. A *malTp1 malTp7* double mutant was constructed, which produced ~30 times more MalT protein than the wild-type strain.

Key words: maltose regulon/*malT*/transcription/translation

Introduction

The maltose regulon of *Escherichia coli* consists of three operons – *malPQ, malEFG,* and *malK lamB* – controlled by a positive regulator gene, *malT* (Débarbouillé and Schwartz, 1979; Raibaud *et al.*, 1979; Silhavy *et al.*, 1979; Raibaud and Schwartz, 1980). Experiments with *malT-lacZ* fusion strains show that the expression of *malT* itself is positively controlled by 3′-5′ cyclic AMP (cAMP) and its receptor, the catabolite activator protein (CAP) (Débarbouillé and Schwartz, 1979). Mutants were isolated, which expressed *malT* at a high level, in the absence as well as the presence of CAP and cAMP (Chapon, 1982). The mutations map close to the beginning of the *malT* cistron. Sequencing data presented here show that some of these mutations are located in the promoter of the *malT* gene, while others are in a region presumed to constitute the ribosome binding site. By combining mutations of the two classes, a strain was constructed that produces ~30 times more MalT protein than the wild-type strain.

Results

Mapping of the mutations by DNA sequencing

Mutations causing increased synthesis of MalT protein, in the absence as well as in the presence of CAP, have been designated *malTp1* to *malTp9* (Chapon, 1982). Their effect on *malT* expression was deduced from that on β-galactosidase synthesis when they were located *cis* to a *malT-lacZ* hybrid gene. Six of them were studied further. Two classes were found (see also Table II). In strains carrying *malTp1, malTp8,* or *malTp9,* the level of β-galactosidase was nearly the same in the presence and absence of CAP, i.e., in a *crp*$^+$ and *crp* background. Conversely, in strains carrying *malTp4, malTp6,* or *malTp7,* the level of β-galactosidase, even though it was already high in a *crp* background, was much higher in a *crp*$^+$ background. The six mutations were transferred to plasmid pOM1, which is derived from pBR322 and carries the *malT* gene (Figure 1) (Raibaud and Schwartz, 1980). It was known (Chapon, 1982) that the mutations mapped between the right end point of deletion *malAΔ511* and that of *malAΔ510* (Figure 2). In addition, because of their effect on *malT* expression, the mutations were expected to map outside the *malT* coding region, i.e., to the left of the initiation codon. In other words, the mutations were presumably located in the first 170 nucleotides at the right end of the *Hpa*II-*Hpa*II fragment shown in Figure 2. This fragment was purified from plasmids carrying the different mutations, and labelled at both ends using polynucleotide kinase and [γ-^{32}P]ATP. Secondary cuts were made with *Hinc*II, and the largest of the two *Hpa*II-*Hinc*II subfragments (Figure 2) was sequenced by the technique of Maxam and Gilbert (1980). The results are shown in Figure 3. The mutations leading to an almost complete insensitivity to the presence of CAP (*malTp1, malTp8,* and *malTp9*) correspond to a GC to TA change 72 base pairs upstream from the initiation codon. The other mutations (*malTp4, malTp6,* and *malTp7*) correspond to an AT to GC change, 12 base pairs upstream from the initiation codon. From their position in the sequence, the former groups, including *malTp1,* could be expected to increase transcription initiation, and the latter, including *malTp7,* to increase translation initiation.

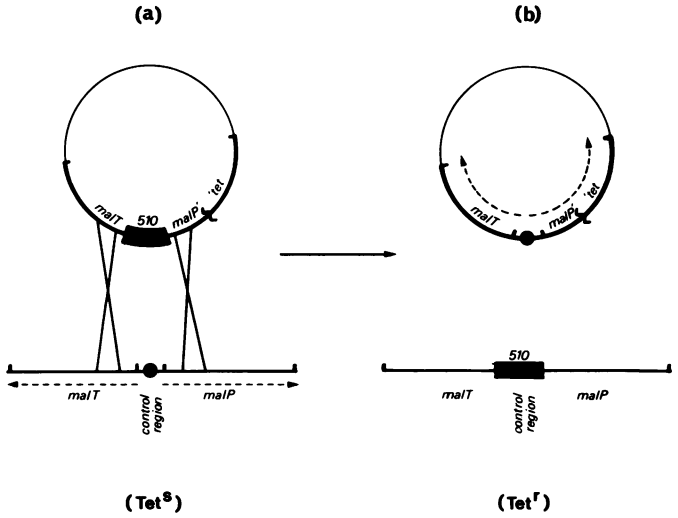

Fig. 1. Transfer of the *malTp* mutations onto plasmid pOM1. The plasmid in **(a)** is a derivative of pOM1 (Raibaud and Schwartz, 1980), which carries deletion *malAΔ510* (Chapon, 1982). The deletion is represented by a filled box. The chromosomal *malT* and *malP* genes are shown below the plasmid, with a *malTp* mutation represented as a dot. The cell shown in **(a)** is Tet^S because deletion *malAΔ510* removed the *malP* promoter, which normally controls the *tet* genes in pOM1. The recombination events shown in **(a)** yield Tet^R cells, which can be selected, and which bear the *malTp* mutation on the plasmid **(b)**.

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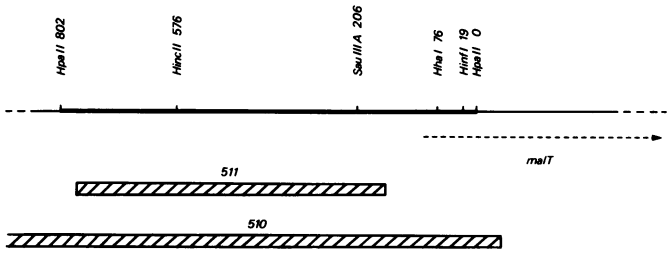

Fig. 2. A DNA segment that contains the *malT* and *malPQ* promoters. The *Hpa*II-*Hpa*II segment shown as a heavy line was previously sequenced (Débarbouillé *et al.*, 1982). The position of a few relevant restriction sites is shown. The numbers are their distance, in base pairs, from the right end of the segment. Deletions *malAΔ511* and *malA510* are shown as hatched bars below the map. The dashed arrows correspond to the beginning of *malT* mRNA as determined in this work (see Figure 4).

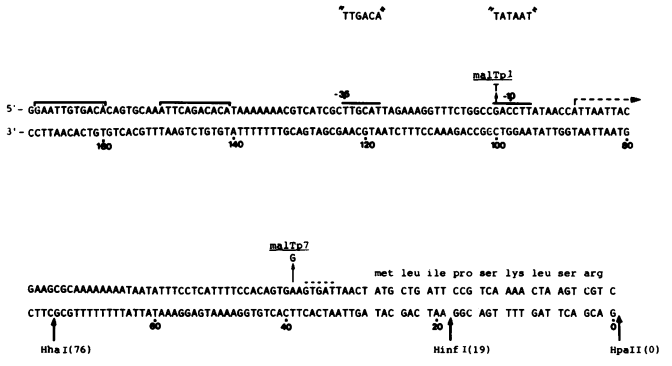

Fig. 3. Location of the *malTp* mutations. The sequence of the right end of the *Hpa*II-*Hpa*II fragment (see Figure 2). The base changes corresponding to *malTp1* and *malTp7* are indicated. The interrupted arrow indicates the beginning of the mRNA (see Figure 4). Heavy lines indicate the position of the so-called −10 (Pribnow box) and −35 regions of the promoter. The consensus sequences (Rosenberg and Court, 1979) for these regions are at the top of the figure. Brackets indicate potential CAP-binding sites, and the interrupted line the Shine and Dalgarno sequence. The numbers under the sequence correspond to the distance expressed in base pairs from the right end of the sequence.

Identification of the transcription start of the malT gene on the nucleotide sequence

The promoter for gene *malT* could not be identified by mere examination of the nucleotide sequence. The fact that strains bearing deletion *malAΔ511* (see Figure 2) still expressed *malT*, simply indicated that the promoter was to the right of this deletion (Débarbouillé *et al.*, 1982). The promoter was precisely mapped by localizing on the sequence the origin of transcription. A specific DNA probe was elongated with reverse transcriptase using total *in vivo* mRNA as a template (Sollner-Webb and Reeder, 1979; Débarbouillé and Raibaud, in preparation). Total RNA was extracted from cells harbouring a multicopy plasmid which carried gene *malT*, or cells harbouring the same plasmid except that the *malT* promoter was deleted. The *malT*-specific mRNA was then allowed to hybridize with a 5′ end-labelled DNA probe (the 19-nucleotide *Hpa*II-*Hin*fI or the 76-nucleotide *Hpa*II-*Hha*I fragments, see Figure 2), and the hybridized DNA probe was extended using reverse transcriptase. The start point of transcription was determined by comparing the length of the extended probe with that of the fragments obtained after chemical cleavage using the Maxam and Gilbert technique. The results with the two DNA probes clearly indicated that

Table I.

E. coli K12 strains	Genotype
pop 3	F⁻ *araD139 ΔlacU169 rpsL relA thi*
pop 2150	pop 3 *malAΔ510*
pop 3931	pop 3 *malTp1*
pop 3951	pop 3 *malT250* [Φ(*malT-lacZ*)542-1 (Hyb) *malTp1*]
pop 3958	pop 3951 *Δcrp39*
pop 3937	pop 3 *malTp7*
pop 3955	pop 3 *malT250* [Φ(*malT-lacZ*)542-1 (Hyb) *malTp7*]
pop 3961	pop 3955 *Δcrp39*
pop 3971	pop 3 *malTp1 malTp7*
pop 3972	pop 3 *malT250* [Φ(*malT-lacZ*)542-1 (Hyb) *malTp1 malTp7*]
pop 3964	pop 3 *malT250* [Φ(*malT-lacZ*)542-1 (Hyb)]
pop 3965	pop 3964 *Δcrp39*
pop 2117	pop 3 pOM 7
pop 2140	pop 3 (pOM 1 *malAΔ510*)

Gene fusions are designated Φ(*malT-lacZ*)542-1 (Hyb). The notation [Φ(*malT-lacZ*) 542-1 (Hyb) *malTp1*] means that the *malTp1* mutation is located *cis* to the *malT-lacZ* hybrid gene. All strains carrying a gene fusion contain also a λ prophage adjacent to the fusion. Pop 2150, pop 2117, and pop 2140 were constructed by Raibaud. Strain pop 3 is the same as strain MC4100 described by Casadaban (1976). The other strains have been previously described (Chapon, 1982) or constructed in this work.

cells harbouring *malT*, on a multicopy plasmid, contained mRNA molecules with 5′ sequences complementary to a DNA segment corresponding to the 89-terminal nucleotides on the *Hpa*II fragment (Figure 4). (Very low amounts of the same mRNA were detected when the plasmid carried a deletion of the *malT* promoter: these residual mRNA molecules were presumably transcribed off the chromosomal copy of the *malT* gene, which was intact). A minor population of mRNA allowed the DNA probes to be extended by 86 instead of 89 terminal nucleotides as shown by the band of weak intensity on the gel (Figure 4). These slightly shorter mRNAs might result from initiation at a secondary origin of transcription of *malT*, but more probably they result either from a specific degradation of *malT* mRNA or staggering by the reverse transcriptase.

From this experiment we concluded that *malTp1* is located 12 nucleotides upstream from the transcription start, i.e., in the Pribnow box (Rosenberg and Court, 1979) of the *malT* promoter, while *malTp7* is located in a transcribed region and may therefore affect translation initiation.

A malTp1 malTp7 double mutant produces ~30 times more MalT protein than wild-type

If the effects of *malTp1* and *malTp7* are exerted at different levels of gene expression, these two mutations should have a cumulative effect when combined together in *cis*. The double mutant was constructed (Figure 5) and a *malT-lacZ* hybrid gene was introduced *cis* to the mutations, as previously described (Débarbouillé and Schwartz, 1979; Chapon, 1982). The resulting strain produced ~30 times more β-galactosidase than the control strain, which contained the same hybrid gene, but no *malTp* mutations (Table II).

The amount of β-galactosidase found in the strain that contained the two mutations *cis* to a *malT-lacZ* hybrid gene was ~25% of that found in a fully induced *lac*⁺ strain (Silverstone *et al.*, 1969). This suggested that the amount of MalT protein synthesized by a *malTp1 malTp7*, but otherwise *malT*⁺ strain, might be sufficient for the protein to be detectable by gel electrophoresis of crude extracts. This is indeed

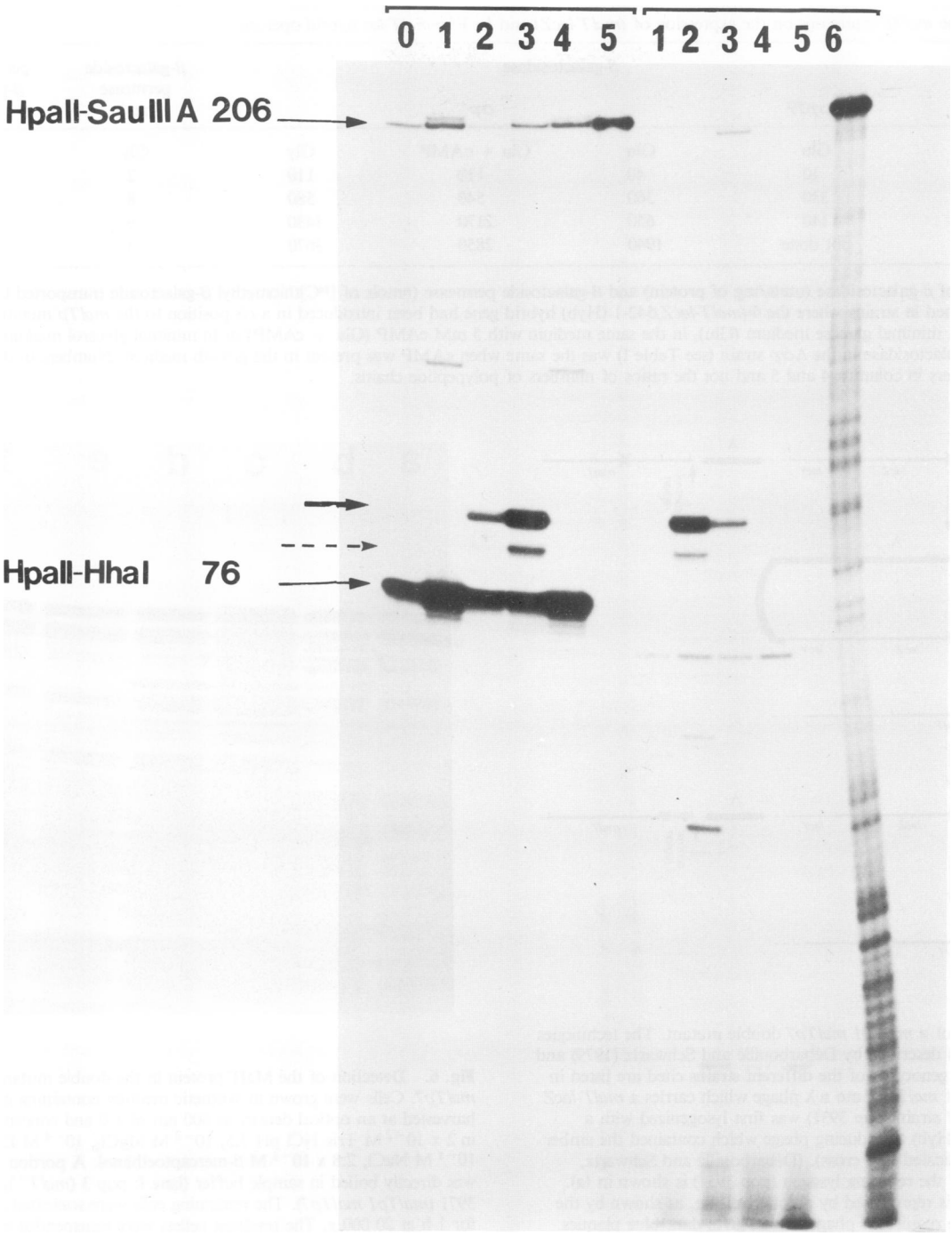

Fig. 4. Reverse transcriptase extension mapping. The hybridization was performed with one of the two 5′end-labelled DNA probes, the 76-bp *Hpa*II-*Hha*I (part **A**) or the 19-bp *Hpa*II-*Hinf*I (part **B**). 50 μg of RNA was used, extracted from strain pop 2117, which harbours plasmid pOM7, carrying the whole *malT* gene but deleted for the *malP* promoter, or from strain pop 2140, which harbours plasmid pOM1, carrying the *malAΔ510* deletion (see Table I). The hybridization was done at 30°C for 3.5 h in the presence of 40% formamide [**lane 1**: pop 2140 (*malT*$^-$), **lane 2**: pop 2117 (*malT*$^+$)], or 80% formamide [**lane 3**: pop 2117 (*malT*$^+$), **lane 4**: pop 2140 (*malT*$^-$)]. The band indicated by a thick arrow corresponds to the DNA probe extended until the start point of transcription of the *malT* gene. The interrupted arrow shows the position of the secondary band discussed in the text. **Lane 5** in **(A)** shows the G sequencing reaction effected on the 206-bp *Hpa*II-*Sau*IIIA fragment (see Figure 3); twice as much of the same material was loaded in **lane 6 (B)**. The two DNA probes were also loaded, the 76-bp *Hpa*II-*Hha*I fragment in **lane 0 (A)**, and the 19-bp *Hpa*II-*Hinf*I fragment which ran out of the gel in **lane 5 (B)**. The positions of the 76-bp *Hpa*II-*Hha*I and the *Hpa*II-*Sau*IIIA fragments are indicated on the figure. The samples were electrophoresed on thin DNA sequencing gel (0.3 mm) containing 8% acrylamide.

the case (Figure 6) while the protein was not detectable in extracts of the single mutant (Chapon, 1982, and unpublished data). The MalT protein in extracts of cells that bore gene *malT* on a multicopy plasmid had been found to be insoluble in the absence of detergent (Raibaud and Schwartz, 1980). The same was true with a *malTp1 malTp7* double mutant. Most of the MalT protein sedimented in 1 h at 20 000 *g*. All strains previously shown to produce increased amounts of MalT protein expressed the maltose operons constitutively, at levels that depended upon the actual concentration of MalT

Table II. Effect of the *malTp* mutations on the expression of *(malT-lacZ)* and *lacY* in *malT-lac* hybrid operons

	β-galactosidase				β-galactoside permease	permease x 1000 / β-galactosidase
	Δcrp39	*crp*$^+$			*crp*$^+$	*crp*
	Glu	Glu	Glu + cAMP	Gly	Gly	Gly
malT$^+$	10	40	110	110	2	18
malTp1	330	360	540	580	8	14
malTp7	140	650	2170	1430	6	4
malTp1 malTp7	not done	1940	2850	3670	13	4

The specific activities of β-galactosidase (units/mg of protein) and β-galactoside permease (nmols of [^{14}C]thiomethyl β-galactoside transported in 20 min by 10^8 cells) were determined in strains where the Φ*(malT-lacZ)*542-1 (Hyb) hybrid gene had been introduced in a *cis* position to the *malTp* mutation (see Table I). Cells were grown in minimal glucose medium (Glu), in the same medium with 5 mM cAMP (Glu + cAMP) or in minimal glycerol medium (Gly). The specific activity of β-galactosidase in the *Δcrp* strain (see Table I) was the same when cAMP was present in the growth medium. Numbers in the last column are ratios of the numbers in columns 4 and 5 and not the ratios of numbers of polypeptide chains.

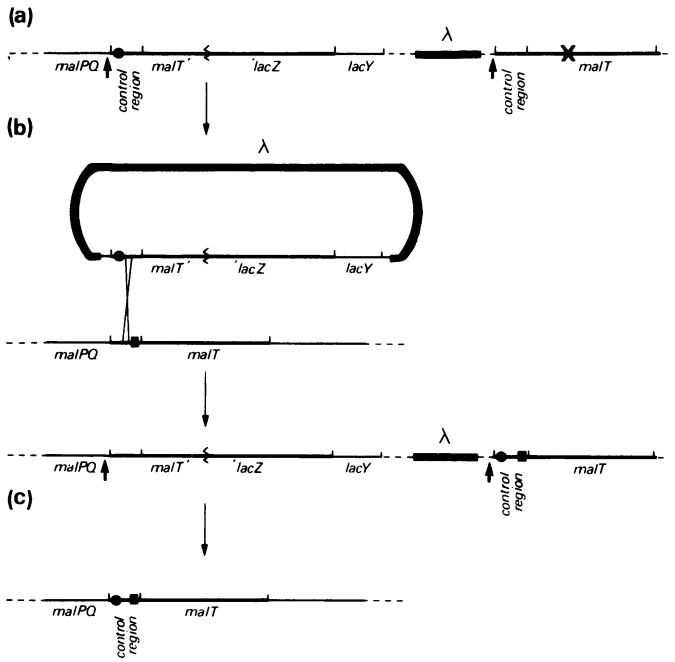

Fig. 5. Construction of a *malTp1 malTp7* double mutant. The techniques used were essentially as described by Débarbouillé and Schwartz (1979) and Hall *et al.* (1982). The genotypes of the different strains cited are listed in Table I. **(a)** Transfer of *malTp1* onto a λ phage which carries a *malT-lacZ* hybrid gene. A *malTp1* strain (pop 3931) was first lysogenized with a λpΦ*(malT-lacZ)*542-1 (Hyb) transducing phage which contained the amber mutation *malT250* (indicated as a cross). (Débarbouillé and Schwartz, 1979). The structure of the resulting lysogen (pop 3951) is shown in **(a)**. The *malTp1* mutation is represented by a dot. Excision, as shown by the thick arrows, yields the required λ phage, which gives dark blue plaques when plated on a Lac$^-$ indicator strain (pop 3) in the presence of the indicator 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal). **(b)** Lysogenization of a *malTp7* strain with the λ*malTp1* phage. The phage constructed as described above was used to infect a *malTp7* strain deleted for the *lac* operon (pop 3937). The *malTp7* mutation is indicated as a dark square. The recombination event shown in **(b)** yields lysogens that express the hybrid gene at the normal low level, and which are therefore pale blue on X-gal containing medium. In lysogens resulting from other recombination events the hybrid gene is controlled by a promoter region containing *malTp1* or *malTp7* and they are dark blue on the same medium. Some of the pale blue colonies were shown to synthesize amylomaltase constitutively at a high level. They were assumed to produce large amounts of MalT protein and to have the structure shown in **(b)**, third line. **(c)** Curing of the prophage. Spontaneous Lac$^-$ segregants of the above strain were obtained. All had lost λ immunity and ~30% of them still expressed amylomaltase constitutively. These were presumed to result from the excision event shown by thick arrows in **(c)** and to carry the *malTp1* and *malTp7* mutations *cis* to a wild-type *malT* gene.

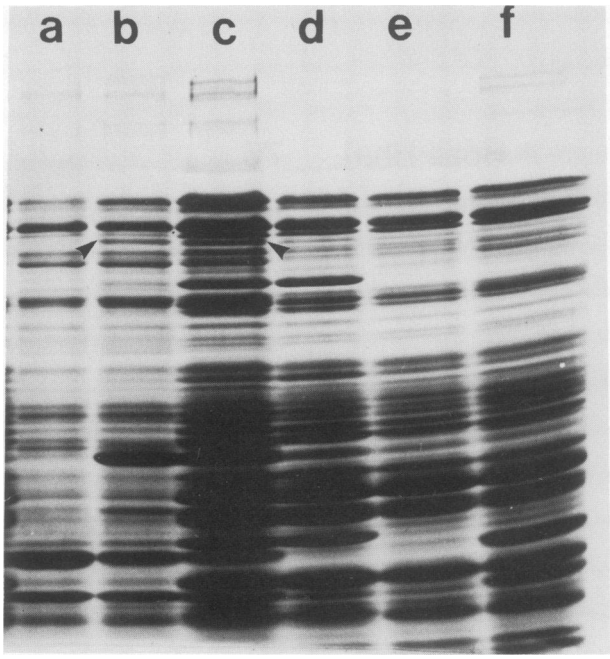

Fig. 6. Detection of the MalT protein in the double mutant strain *malTp1 malTp7*. Cells were grown in synthetic medium containing glycerol, harvested at an optical density at 600 nm of 1.0 and concentrated 20-fold in 2 x 10^{-2} M Tris HCl pH 7.5, 10^{-2} M $MgCl_2$, 10^{-4} M EDTA, 5 x 10^{-2} M NaCl, 2.8 x 10^{-3} M β-mercaptoethanol. A portion of the cells was directly boiled in sample buffer (**lane f**: pop 3 (*malT*$^+$), **lane c**: pop 3971 (*malTp1 malTp7*). The remaining cells were sonicated and centrifuged for 1 h at 20 000 *g*. The resulting pellets were resuspended in the same volume as that of the supernatant in the buffer described above. The supernatant (**lane c**: pop 3, **lane d**: pop 3971) and the pellet (**lane a**: pop 3, **lane b**: pop 3971) were boiled in sample buffer. The samples were analysed on 10% polyacrylamide gels in the presence of SDS as previously described (Débarbouillé *et al.*, 1978). The position of the MalT protein (Débarbouillé *et al.*, 1982) is indicated by an arrow.

protein in the cell (Débarbouillé and Schwartz, 1980). Strains bearing *malTp1* or *malTp7* synthesized amylomaltase (*malQ* product) constitutively only at ~25% of the fully induced level (Chapon, 1982). In the double mutant, on the other hand, amylomaltase synthesis was fully constitutive (not shown). This result, which was predictable, was used in a plate test during the construction of the double mutant (see Figure 5).

Effect of malTp1 and malTp7 on lacY expression in malT-lac hybrid operons

A mutation affecting transcription and a mutation affecting translation might be expected to have different effects in the expression of a polycistronic operon. This could be tested in stains containing *malTp1* or *malTp7 cis* to a *malT-lacZ* hybrid gene, because these strains contained *lacY*, the gene coding for β-galactoside permease, as part of the same operon as the hybrid gene (Table II). Mutation *malTp1*, which is believed to increase the rate of transcription initition, increased *lacY* expression by approximately the same factor as *malT-lacZ* expression. However, *malTp7*, which is assumed to increase the rate of translation initiation, had much less effect on *lacY* expression than on β-galactosidase synthesis.

Discussion

The six mutations we studied, which increase *malT* expression, were obtained independently. However, they all correspond to one or other of two base changes. This result may indicate that only few base changes, and perhaps only these two, can lead to a significant increase in *malT* expression.

Mutation *malTp1* and the other mutations of the same base change increase the rate of transcription initiation at the *malT* promoter. Two lines of evidence supported this conclusion. First, mutation *malTp1* rendered the expression of *malT* nearly independent of CAP, and CAP is known to act at the level of transcription (Pastan and Adhya, 1976; Simpson, 1980; Ullmann and Danchin, 1982). Secondly, *malTp1* is located 12 nucleotides upstream from the transcription start, i.e., in the Pribnow box (Rosenberg and Court, 1979; Siebenlist *et al.*, 1980) of the *malT* promoter. The replacement of a G by a T at this position results in the formation of a nucleotide hexamer (TACCTT) whose sequence is much closer to that of the "consensus" Pribnow box (TATAAT) than is that of the wild-type hexamer (GACCTT), the first T in the consensus sequence being one of the most conserved bases. We conclude that the *malT* promoter is intrinsically relatively weak, because of an inadequate Pribnow box. The binding of RNA polymerase to this promoter would be enhanced by the CAP-cAMP complex. Mutation *malTp1* would allow quasi-optimum binding of RNA polymerase to the promoter, such that it could be only slightly enhanced by CAP and cAMP. Mutations similar to *malTp1* have been described in the *lac* (Arditti *et al.*, 1973; Reznikoff and Abelson, 1978; Maquat and Reznikoff, 1980) and *ara* (Horwitz *et al.*, 1980) systems.

The nature and location of the base change corresponding to *malTp7* strongly suggest that this mutation leads to an increase in the rate of translation initiation by allowing a more efficient binding of the ribosomes to *malT* mRNA. As a rule procaryotic cistrons are preceded by a short sequence, the "Shine and Dalgarno sequence", which is complementary to the 3′ end of 16S rRNA and which is an important element of the ribosome binding site (Shine and Dalgarno, 1975; Gold *et al.*, 1981). Gene *malT* is preceded by such a sequence, which, however, is unusual in that it is mainly complementary to nucleotides 8 – 12 from the 3′ end of 16S rRNA, while the sequence preceding other cistrons is usually complementary to nucleotides located closer to the 3′ end of the rRNA (Figure 7).

Mutation *malTp7* resulted in the creation of a GGAG sequence, which is complementary to the sequence extending from the fourth to the seventh nucleotide from the 3′ end of

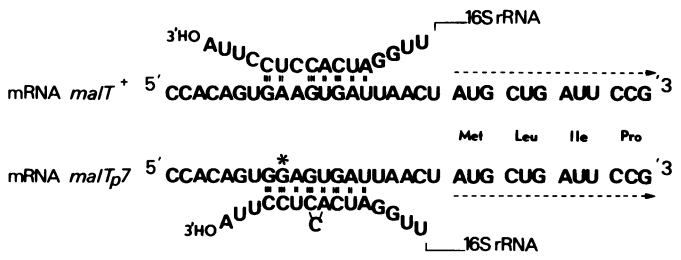

Fig. 7. Potential ribosome binding sites on *malT*⁺ and *malTp7* mRNAs. Complementarities between the 3′-terminal sequence of the 16S rRNA and the mRNA sequence of a *malT*⁺ or *malTp7* mRNA are shown. The base substitution (A→G) resulting from mutation *malTp7* is indicated on the mRNA sequence by an asterisk. The 3′-terminal sequence of 16S rRNA is shown above the *malT*⁺ mRNA or below the *malTp7* mRNA and the base pairing possibilities are indicated. The N-terminal amino-acid sequence of the MalT protein is shown between the mRNA sequences (Débarbouillé *et al.*, 1982).

16S rRNA. The distance of this GGAG sequence from the initiation codon (nine nucleotides) is well within the range of distances found in other systems (Gold *et al.*, 1981). In mutant *malTp7*, there is an almost complete complementarity of the mRNA with a sequence extending from the fourth to twelfth nucleotide of the 3′ end of 16S rRNA. Therefore, we conclude that the ribosome binding site located at the beginning of the *malT* cistron is normally rather weak, and that *malTp7* resulted in the formation of a much stronger binding site, allowing more efficient initiation of translation. This conclusion is clearly compatible with the fact that *malT* expression is still controlled by CAP in a *malTp7* mutant.

The finding that *malT* expression could be increased by mutations affecting either the promoter or the ribosome binding site was unexpected. Differential gene expression in bacteria is generally assumed to be mainly the result of different promoter efficiencies. Mutations leading to an increase in the expression of a particular gene are usually considered to be "up promoter" mutations. Mutations leading to an increased expression of the *malT* gene (Débarbouillé and Schwartz, 1980) had been assumed to be located in the promoter. These mutations have not been mapped by DNA sequencing but, since they still allow *malT* expression to be controlled by CAP, they may very well be located, like *malTp7*, in the ribosome binding site.

A limitation of gene expression at the translation level offers one advantage for genes that are expressed at a very low rate: it provides a more regular synthesis of the gene product. In a wild-type strain there may be of the order of a hundred to a few hundred molecules of MalT polypeptide (Débarbouillé and Schwartz, 1979). If *malT* mRNA is translated at a high rate, the synthesis of one or two mRNA molecules per generation could suffice to produce such a concentration of MalT protein (Kennel and Riezman, 1977). Statistically this would result in significant variations of the concentration of MalT protein in individual cells. Concomitant variations in the expression of the *mal* operons would occur (Débarbouillé and Schwartz, 1980) and this would presumably be undesirable. Such a situation can be avoided by having a higher rate of transcription, but a lower rate of translation. Similar reasoning could be applied to the case of gene *CI* in the λ prophage. This gene is also transcribed at a reasonable rate, but poorly translated, presumably because the mRNA starts with AUG and therefore does not contain a Shine and Dalgarno sequence (Ptashne *et al.*, 1976; Scherer *et al.*, 1980). In that

case the importance of having a smooth synthesis of the gene product is even more obvious.

Mutations *malTp1* and *malTp7* were recombined into a hybrid *malT-lac* operon, which contained a *malT-lacZ* hybrid gene as the promoter proximal gene, and *lacY*, as a distal gene. Mutation *malTp1* resulted in a parallel increase (~5-fold) in the expression of the two genes, as would be expected for a mutation leading to an increase in transcription initiation. Mutation *malTp7*, on the other hand, led to a greater increase in the expression of the proximal gene (14-fold) than of the distal gene (3-fold). Therefore, the expression of a distal gene is not strictly correlated with the level at which the proximal gene is translated. A similar conclusion was recently drawn from studies of a *lamB-lac* hybrid operon (Schwartz *et al.*, 1981). Further investigations on this point may shed additional light on the mechanism of polarity in operon expression.

A strain that contained both *malTp1* and *malTp7 cis* with respect to an otherwise wild-type *malT* gene produced ~30 times more MalT protein than a wild-type strain. For reasons that are not understood, this is somewhat lower than would be expected from a strictly cumulative effect of the two mutations. Unlike strains that harboured a *malT* gene on a multicopy plasmid (Raibaud and Schwartz, 1980) the double mutant did not show any obvious growth defect. It expressed the maltose operons constitutively and was, therefore, phenotypically similar to previously described $malT^c$ mutants (Débarbouillé *et al.*, 1978). In the latter case, however, the mutations were located within the coding sequence and presumably affected the structure, rather than the synthesis, of MalT protein.

Materials and methods

Strains and media

The strains of *E. coli* K12 used are listed in Table I. All growth media [complete medium (ML) and synthetic medium (M63)] were previously described (Chapon, 1982). Ampicillin was used at 100 μg/ml in solid or liquid media. Tetracycline, unless otherwise stated, was used at 5 μg/ml in liquid media and 10 μg/ml in solid media.

Enzyme assays

β-Galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) was assayed according to Miller (1972). The *lacY* product (β-galactoside permease) was assayed by measuring the accumulation of ^{14}C-labelled thiomethyl β-D-galactoside (Rickenberg *et al.*, 1956). The plate test for detection of clones synthesizing amylomaltase constitutively was as described by Débarbouillé *et al.* (1978).

Transfer of the mutations from the chromosome to the plasmid

The techniques used were almost exactly as described by Raibaud and Schwartz (1980). Genetic mapping had shown that the *malTp* mutations fail to recombine with deletion *malAΔ510* (Chapon, 1982). They were transferred from the chromosome to plasmid pOM1 (Raibaud and Schwartz, 1980) using the selection described in Figure 1. However, the scheme shown in the figure represents a simplification of the actual events, especially because the cells contain several copies of the plasmids. Most of the plasmids extracted from the Tet^R clones still carried the deletion. To purify those that harboured the *malTp* mutation, the plasmids extracted from the Tet^R clones were used to transform a *malT* derivative of C600. The Tet^R transformants were selected on ML agar containing 20 μg/ml tetracycline. (With lower concentrations of tetracycline the transformants still contained a mixture of plasmids carrying the *malTp* mutation and plasmids carrying the deletion. This is probably the result of the toxicity of plasmids that express both *malT* and the *malP-tet* hybrid operon). The plasmids extracted from the Tet^R transformants gave the same digestion pattern with *Hin*cII as observed with pOM1. The presence of the *malTp* mutations on the plasmids was verified by transferring the mutations back to the chromosome, by a procedure the reverse of that shown in Figure 1 with selection for a Mal^+ phenotype. Since the curing of the resulting strains was usually difficult, the phenotype, i.e., partially constitutive amylomaltase synthesis (Débarbouillé *et al.*, 1978) was studied after transducing the *malT* gene into a plasmid-free strain (pop 2150, Table I).

DNA sequence determination

The 5′-end labelled DNA fragments were prepared essentially as described by Débarbouillé *et al.* (1982). The DNA was sequenced by the method of Maxam and Gilbert (1980). The end-labelled fragments, obtained after the chemical degradations, were analysed by electrophoresis on thin gels (0.3 mm) containing 7% acrylamide (Sanger and Coulson, 1978).

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