mal Regulon

Maltose Operon

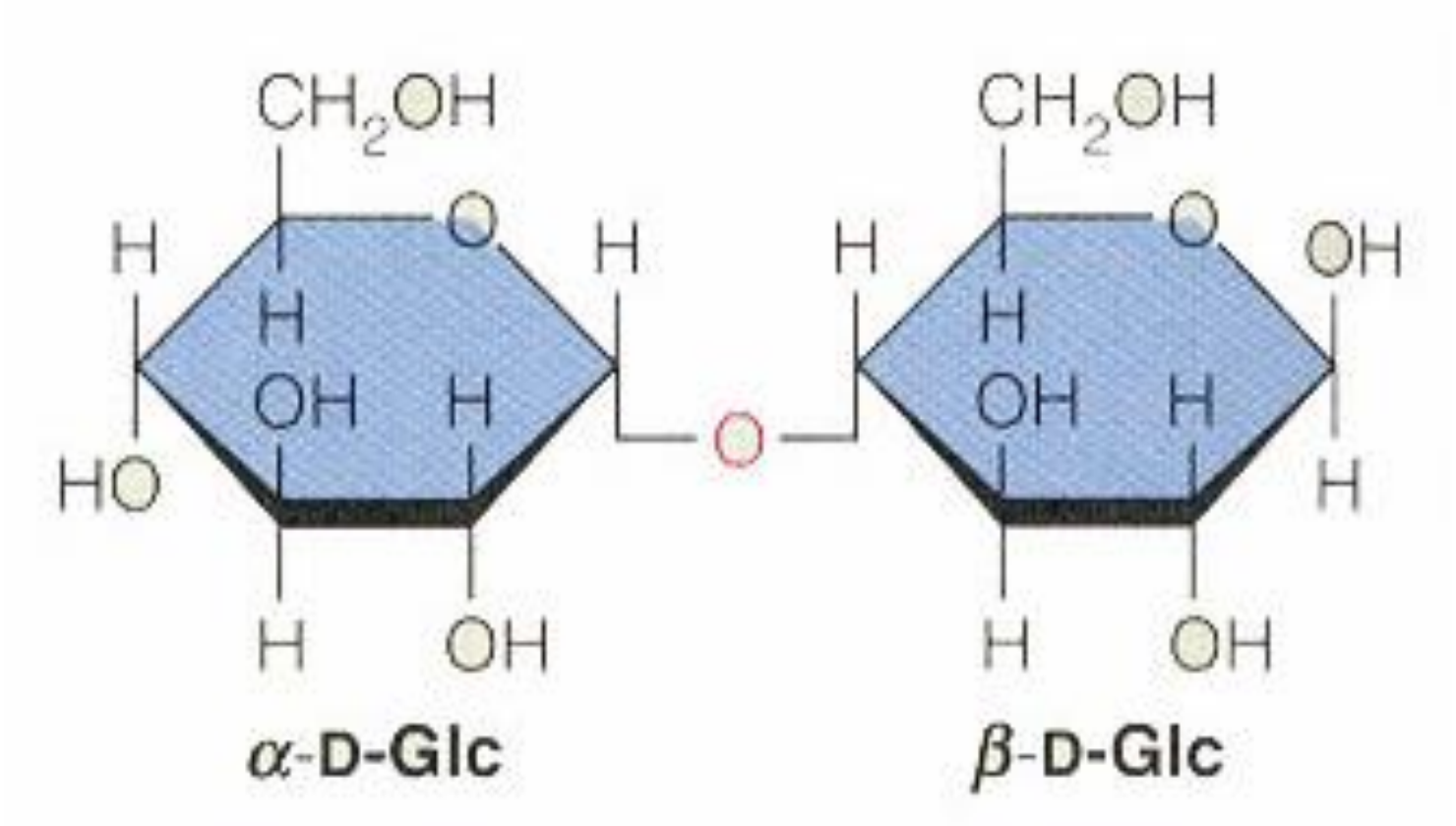


TABLE 1. *mal* genes and their products, genes controlling *mal* gene expression, and genes related to maltodextrin metabolism

Gene	Position on chromosome (min)	Gene product and function
mal genes and their main regulator		
malT	76.5	Transcriptional activator, essential for transcription of all *mal* genes except the *malI/X/Y* gene cluster. Binds ATP and maltotriose as inducer.
malE	91.4	Periplasmic MBP; binds maltose/maltodextrins with micromolar affinity.
malF	91.4	Intrinsic membrane protein of the transport system. In association with MalG and MalK, it forms the $MalFGK_2$ translocation complex.
malG	91.4	Intrinsic membrane protein of the transport system. In association with MalF and MalK, it forms the $MalFGK_2$ translocation complex.
malK	91.5	Transport ATPase, responsible for energization of transport. In association with MalF and MalG, it forms the $MalFGK_2$ translocation complex. Target of inducer exclusion by unphosphorylated $EIIA^{Glc}$ of the PTS. In the absence of inducer, it interacts with MalT to cause repression.

lamB	91.5	Receptor for phage λ and specific pore for maltodextrins (maltoporin, glycoporin).
malM	91.5	Periplasmic protein of unknown function, partially associated with the outer membrane. Contains an Ala-Pro linker also found in OmpA.
malP	76.5	Maltodextrin phosphorylase. Substrates are maltopentaose and larger maltooligosaccharides. *malP* mutants still grow on maltose but accumulate large amount of maltodextrins under these conditions.
malQ	76.4	Amylomaltase. Maltodextrinyltransferase with maltotriose as the smallest substrate. *malQ* mutants cannot grow on maltose, are sensitive to maltose, and are constitutive for *mal* gene expression.
malS	80.5	Periplasmic α-amylase, cleaves preferentially maltohexaose from the nonreducing end of meltotextrins.
malZ	9.1	Maltodextrin glucosidase and γ-cyclodextrinase, cleaves glucose sequentially from the reducing end of maltodextrins. Maltotriose is the smallest substrate. It linearizes γ-cyclodextrin but not α- and β-cyclodextrin.

Genes whose products control *mal* gene expression		
cya	85.9	Adenylate cyclase. Production of cAMP, involvement in catabolite repression.
crp	75.1	cAMP-binding protein, needed for the transcription of *malT* and the transport gene cluster.
malI	36.6	Repressor for *malXY*, not dependent on MalT, inducer unknown.

Gene	Position on chromosome (min)	Gene product and function
malX	36.6	Enzyme II of the PTS, transports and phosphorylates glucose, can transport maltose by diffusion.
malY	36.6	βC-S lyase (cystathionase). Overproduction reduces *mal* gene expression by interaction with MalT and its inactivation.
aes (*ybaC*, *orf203*)	10.8	Esterase. Overproduction reduces *mal* gene expression, presumably by interaction with MalT and its inactivation.
mlc	35.9	Gene regulator, represses the expression of *malT* and *manXYZ*.
maa (*mac*, *F183a*)	10.32	Glucose/maltose transacetylase, not MalT dependent, responsible for exit of maltose and glucose in their acetylated forms.

Genes whose products affect endogenous synthesis of inducer		
glgA	75.4	Glycogen synthase. ADP-dependent synthesis of glycogen. Degradation of glycogen yields maltotriose, which, in *malQ* mutants, leads to constitutivity of the maltose system. *glgA* mutants have a lower level on uninduced *mal* gene expression than *glgA*$^+$ strains.
glgC	75.4	ADP-glucose-pyrophosphorylase. Synthesis of ADP-glucose, needed for constitutive *mal* gene expression in *malQ* mutants. *glgC* mutants have a lower uninduced *mal* gene expression than *glgC*$^+$ strains.
glgP	75.4	Glycogen phosphorylase. Glycogen degradation and formation of glucose-1-phosphate. Possibly involved in synthesis of endogenous inducer.
glgB	75.4	Branching enzyme
glgX	75.4	Amylase-like enzyme, role in glycogen degradation unclear.
amyA	43.2	Cytoplasmic α-amylase, not MalT dependent, no apparent role in glycogen degradation.

galU	27.8	UDP-glucose pyrophosphorylase. Possible origin of cytoplasmic unphosphorylated glucose.
glgS	68.7	Short polypeptide, involved in RpoS-dependent glycogen synthesis.
glk	54.0	Glucokinase. Reduces level of internal glucose which can form endogenous inducer, responsible for *mal* gene repression at high osmolarity.
treR	96.2	Repressor for *treB* and *treC*. *treR* mutants allow transport of maltose via the *treB*-encoded transport system and induce *treC*, whose product is involved in inducer synthesis.
treB	96.1	Enzyme II for trehalose of the PTS, allows transport of maltose.
treC	96.1	Trehalose-6-phosphate hydrolase, involved in inducer synthesis.

Gene	Position on chromosome (min)	Gene product and function
pgm	15.4	Phosphoglucomutase. Needed for the synthesis of endogenous inducer. A *pgm* mutant can still grow on maltose but only in the presence of MalZ.
Genes that affect *mal* gene expression by an unknown mechanism when mutated		
asuE	25.6	tRNA-modifying enzyme. An *asuE* mutant increases *mal* gene expression at high osmolarity.
yjeA (*genX*)	94.4	Homolog to lysyl-tRNA synthases LysS and LysU. A *genX* mutant interferes with the ability of a *malQ malZ292 pgm* strain to grow on maltose.
envZ	76.1	Sensor kinase of the two-component osmoregulatory system. Certain *envZ* mutants that lead to the overphosphorylation of OmpR show reduced *malT* expression.
phoP phoQ	25.7	Two-component system responding to Mg^{2+} starvation. Overexpression of the response regulator leads to *mal* gene repression.

1. *mal* regulon regulated by CRP

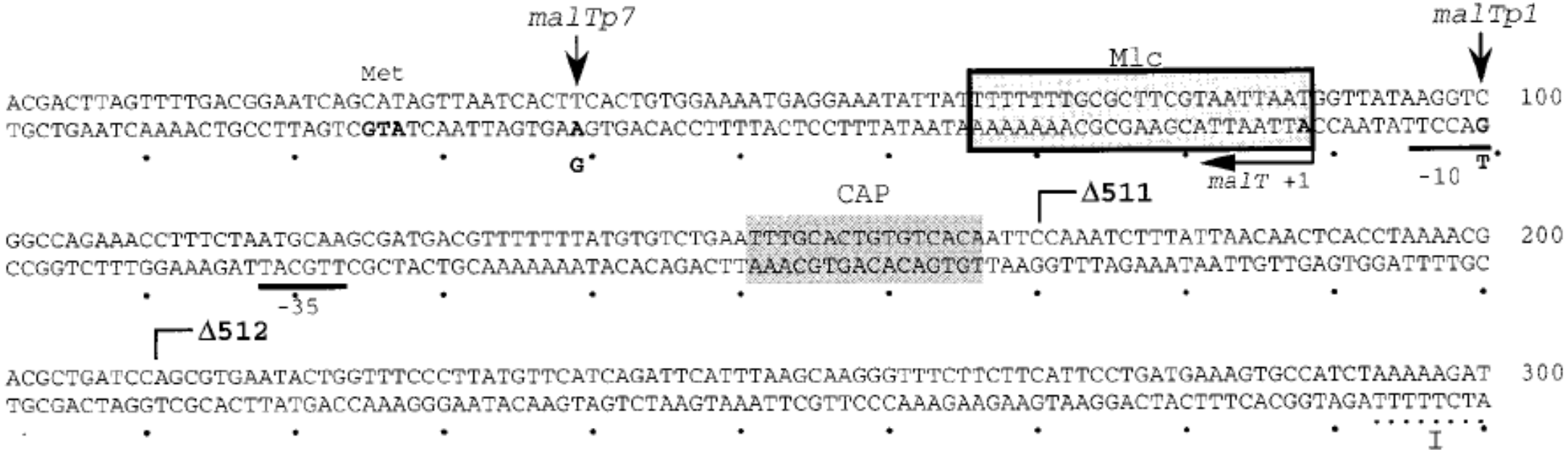
2. MalT also regulates the *mal* promoters

-requires ATP

-activated by inducer (maltotriose)

-Some *mal* promoters *mal*E*p* & *mal*K*p* use both CRP and MalT

FIG. 1. Regulatory region upstream of *malT*. The unboxed shaded area represents the cAMP/CAP binding site for the *malT* promoter. The boxed shaded area represents the binding site of Mlc, the regulator of *malT* expression, as determined by footprint analysis. The arrows at *malT* +1 and +1 *malP* indicate the transcriptional startpoints of the *malT* and *malP* transcripts, respectively. The arrows upstream of the *malP* promoter show the single and tandem MalT boxes needed for *malP* expression. −10 and −35 regions are indicated for the *malT* promoter only. The changes of AT to GC at position 39 (*malTp7*) and of GC to TA at position 100 (*malTp1*) are mutations that increase *malT* expression at the translational and transcriptional levels, respectively. *malTp1* renders *malT* expression independent of the cAMP/CAP complex (34). The extent of the deletions upstream of the *malT* promoter, Δ511, Δ512, Δ513, and Δ514, are indicated. Their *malT* expression relative to the wild type is increased by a factor of 2.8 (Δ511), 2.2 (Δ512), 1.1 (Δ513), and 1.0 (Δ514) (200). The Tn*10*(Cam) insertion at nucleotide 418 leads to a 2.5-fold increase in *malT* expression. Dotted lines indicate homology to sites that have been identified as OmpR binding sites. In particular, I and III indicate homology to site FII and II indicates site FI as defined in reference 155. Modified from reference 200 with permission of the publisher.



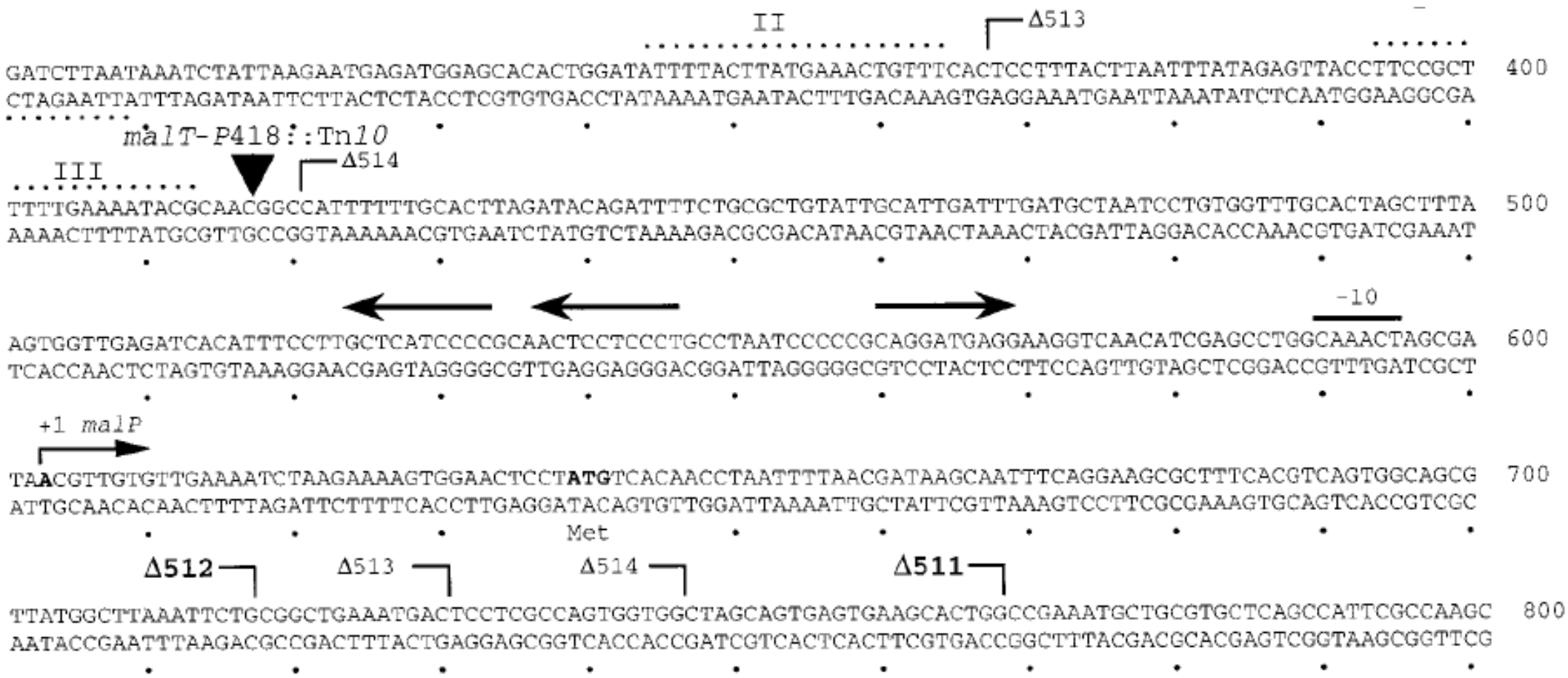
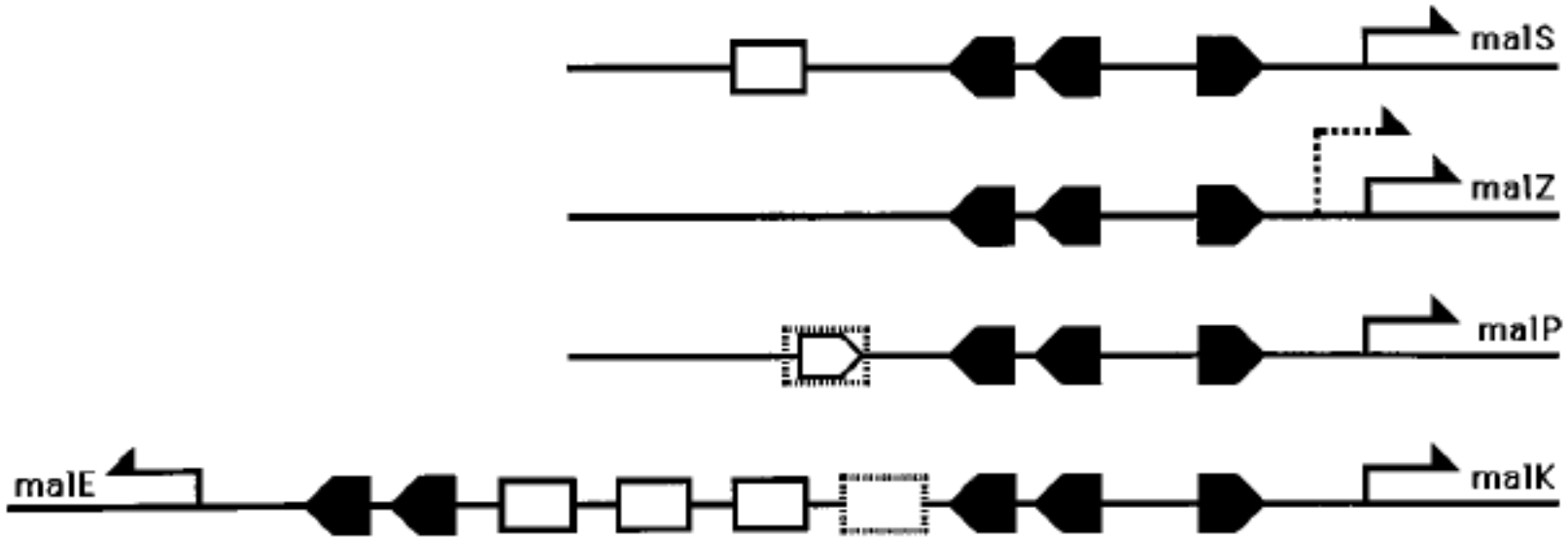
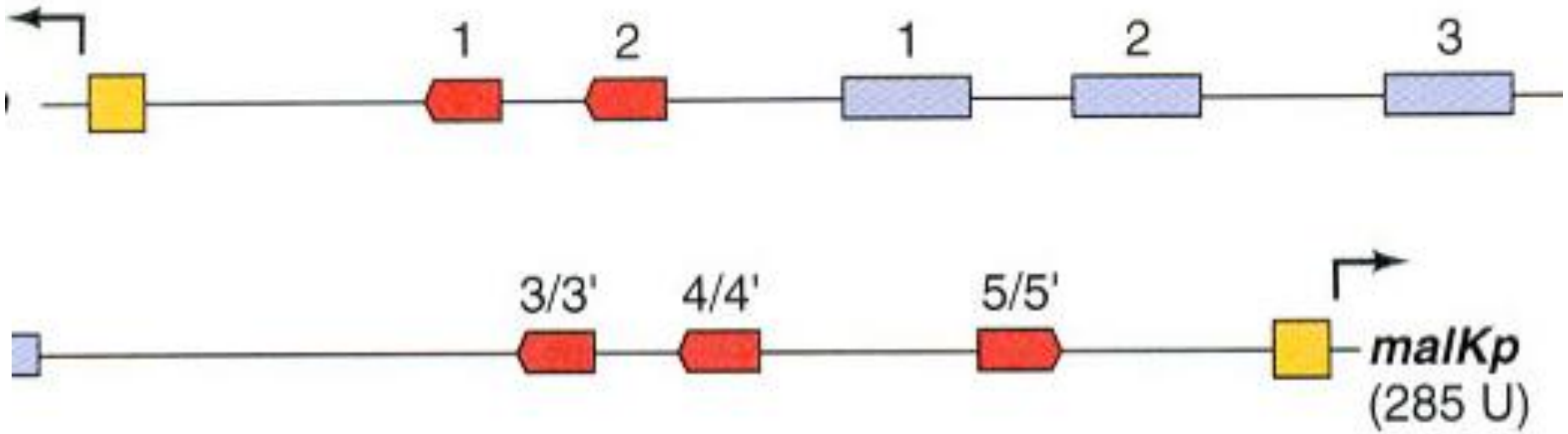

II
Δ513
GATCTTAATAAATCTATTAAGAATGAGATGGAGCACACTGGATATTTTACTTATGAAACTGTTTCACTCCTTTACTTAATTTATAGAGTTACCTTCCGCT 400
CTAGAATTATTTAGATAATTCTTACTCTACCTCGTGTGACCTATAAAATGAATACTTTGACAAAGTGAGGAAATGAATTAAATATCTCAATGGAAGGCGA
malT-P418::Tn10
III
Δ514
TTTTGAAAATACGCAACGGCCATTTTTTGCACTTAGATACAGATTTTCTGCGCTGTATTGCATTGATTTGATGCTAATCCTGTGGTTTGCACTAGCTTTA 500
AAAACTTTTATGCGTTGCCGGTAAAAAACGTGAATCTATGTCTAAAAGACGCGACATAACGTAACTAAACTACGATTAGGACACCAAACGTGATCGAAAT
-10
AGTGGTTGAGATCACATTTCCTTGCTCATCCCCGCAACTCCTCCCTGCCTAATCCCCCGCAGGATGAGGAAGGTCAACATCGAGCCTGGCAAACTAGCGA 600
TCACCAACTCTAGTGTAAAGGAACGAGTAGGGGCGTTGAGGAGGGACGGATTAGGGGGCGTCCTACTCCTTCCAGTTGTAGCTCGGACCGTTTGATCGCT
+1 malP
TAACGTTGTGTTGAAAATCTAAGAAAAGTGGAACTCCTATGTCACAACCTAATTTTAACGATAAGCAATTTCAGGAAGCGCTTTCACGTCAGTGGCAGCG 700
ATTGCAACACAACTTTTAGATTCTTTTCACCTTGAGGATACAGTGTTGGATTAAAATTGCTATTCGTTAAAGTCCTTCGCGAAAGTGCAGTCACCGTCGC
Met
Δ512
Δ513
Δ514
Δ511
TTATGGCTTAAATTCTGCGGCTGAAATGACTCCTCGCCAGTGGTGGCTAGCAGTGAGTGAAGCACTGGCCGAAATGCTGCGTGCTCAGCCATTCGCCAAGC 800
AATACCGAATTTAAGACGCCGACTTTACTGAGGAGCGGTCACCACCGATCGTCACTCACTTCGTGACCGGCTTTACGACGCACGAGTCGGTAAGCGGTTCG

FIG. 2. Scheme for the promoter structures of the different *mal* operons. Arrows indicate transcriptional start points. Solid large arrow-shaped bullets represent MalT binding sites (MalT boxes), and open rectangles represent cAMP/CAP binding sites. The open arrow-shaped bullet indicates a MalT box that has been identified by footprinting but seems dispensable for Mal-dependent transcriptional activation of *malP* (46). This site overlaps a CAP binding site (dashed cAMP/CAP binding site) as determined by footprint analysis. Again, mutation of this site does not affect the transcription of *malP* (46). Similarly, the cAMP/CAP binding site most proximal to the *malK* promoter (dashed rectangle) has been identified by footprint analysis but is not essential for transcription of *malK* (279). Having two MalT binding sites in a direct repeat is a recurrent feature of *mal* promoters and plays a crucial role in their activation (281). The dashed arrow in the *malZ* promoter indicates a second transcriptional start site that is independent of MalT and more frequently used at high osmolarity. The cAMP/CAP binding site in the *malS* promoter has not been tested for its functional importance. The three cAMP/CAP binding sites between *malK* and *malE* are essential for the transcription of both genes (279).



The *mal*E*p* & *mal*K*p* region

*mal*E*p* (divergent operons)

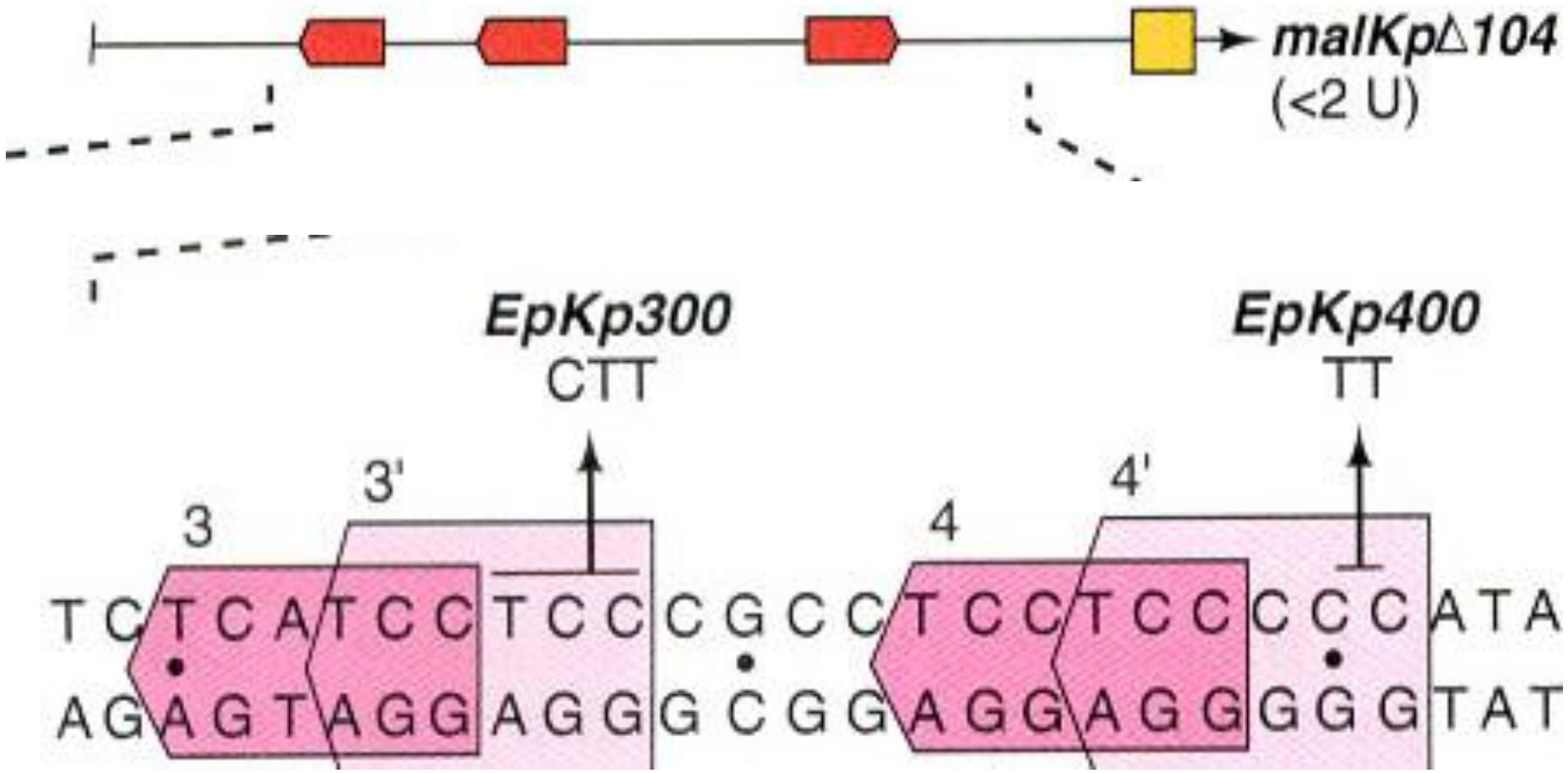


-2 operons transcribed in opposite directions (3 genes each)

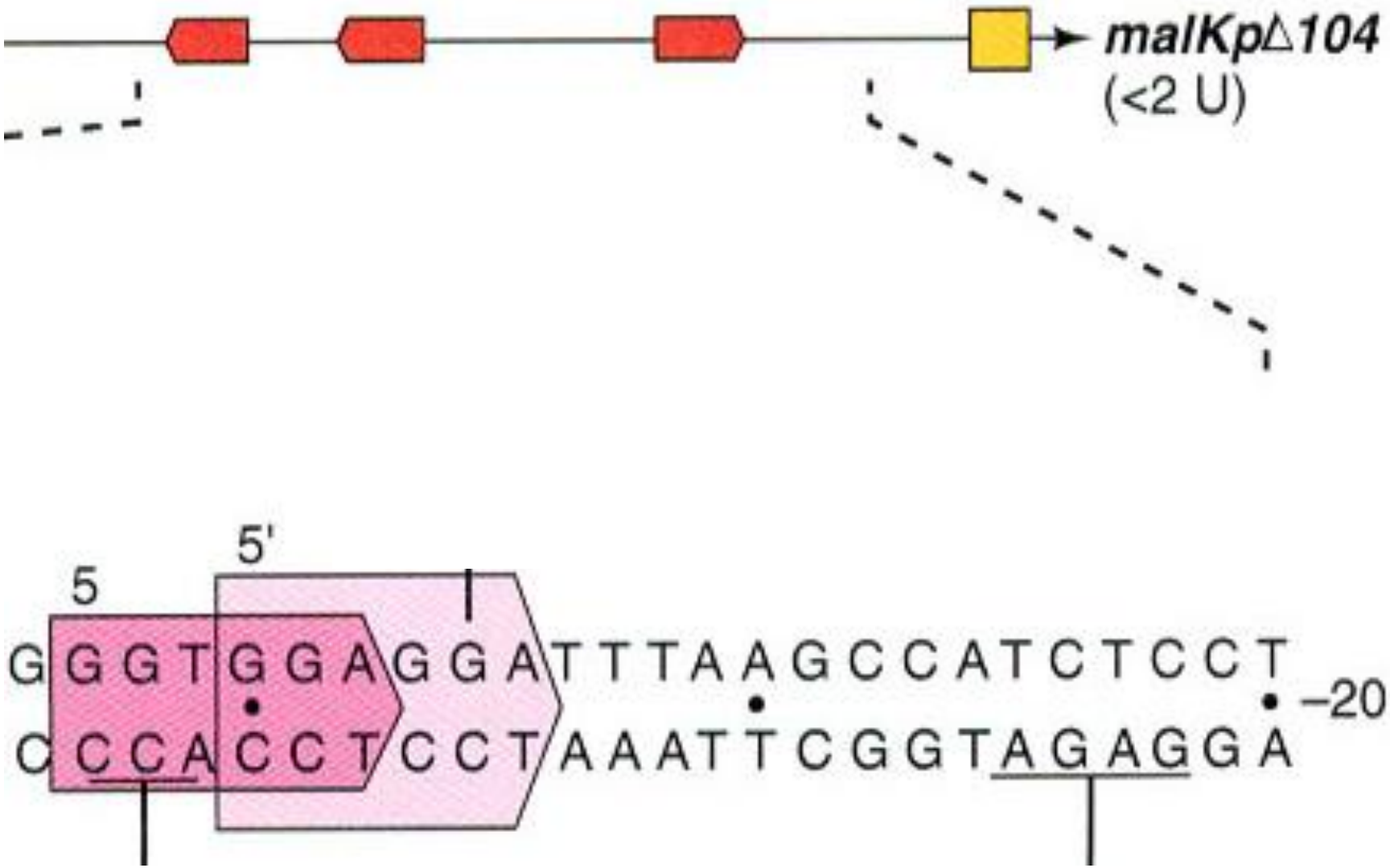
-3 CRP binding sites & 5 MalT binding sites

The MalT Binding Sites

-each site consists of 2 6-bp overlapping binding regions



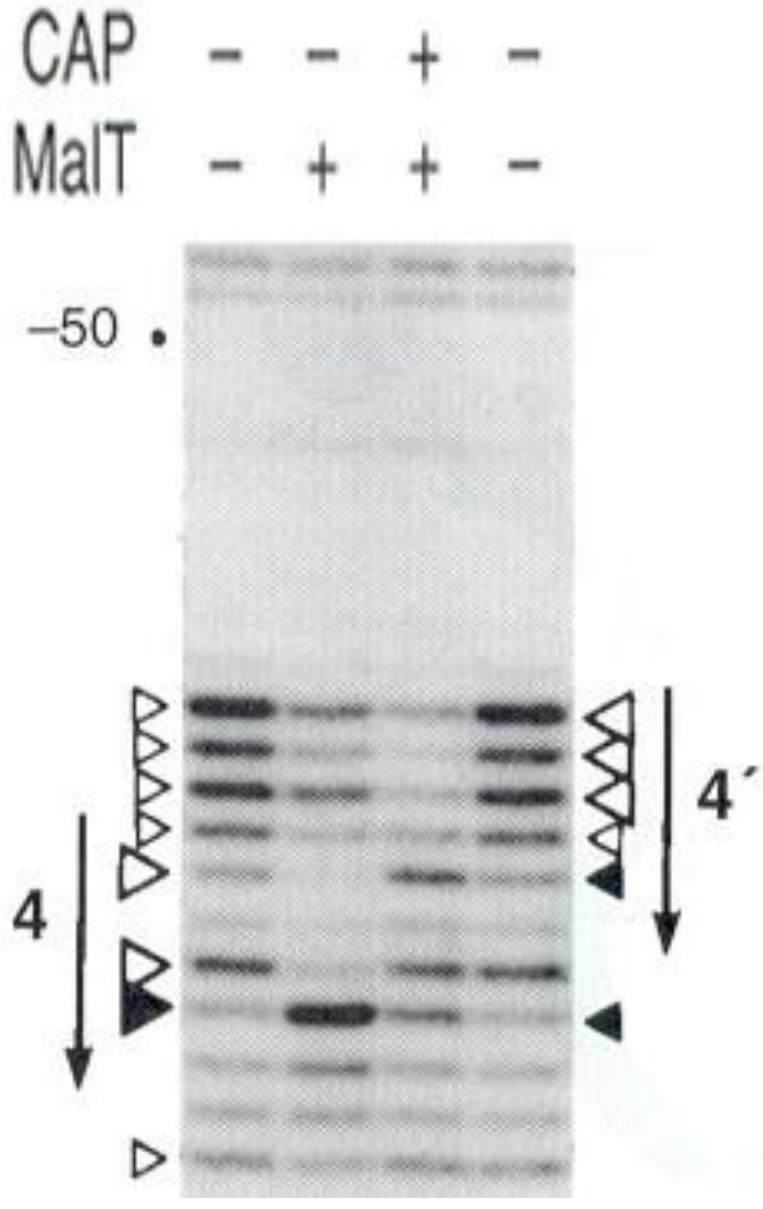
-the third site

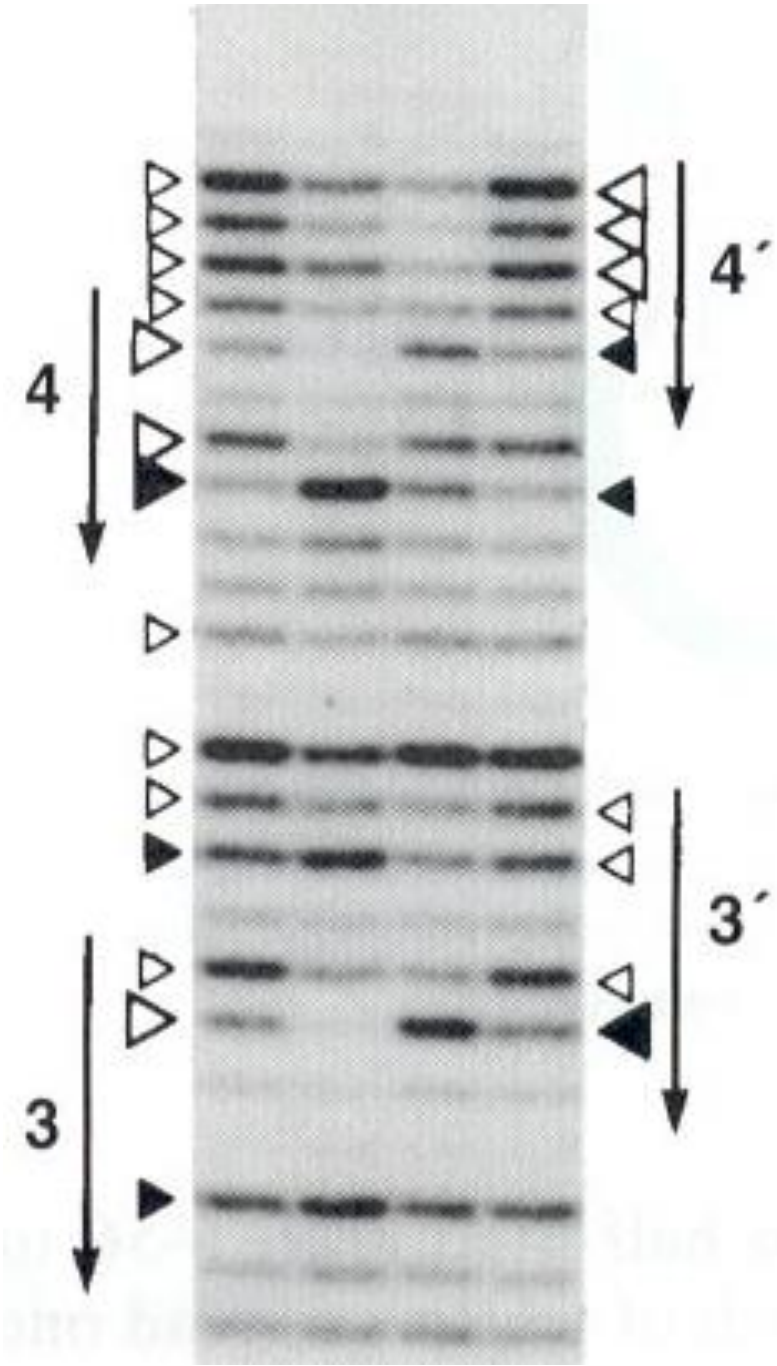


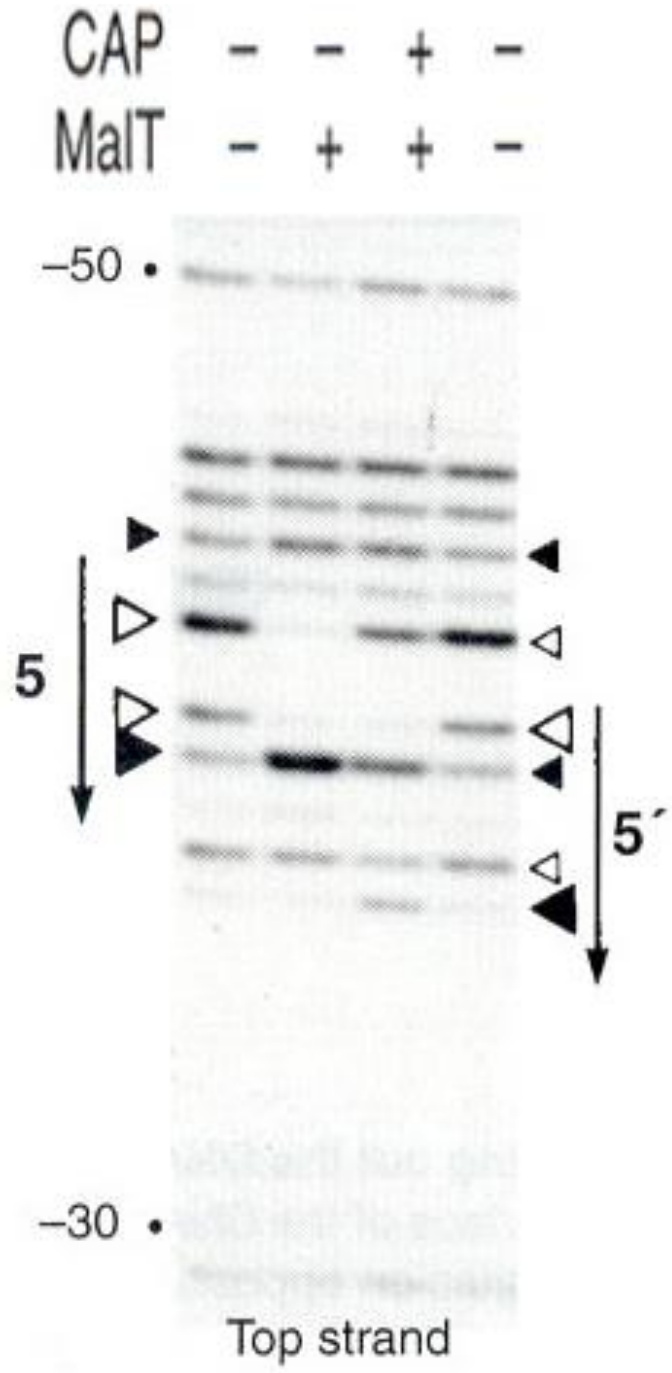
DNA footprinting showing 3-bp shift in MalT binding after CRP (CAP) binding

-MalT has higher affinity for sites 3, 4, and 5 than for sites 3’, 4’, and 5’.

-sites 3,4, and 5 are exactly 3-bps short of maximal spacing for promoting RNA polymerase binding.




4
4´
3
3´


CAP – – + –
MalT – + + –
–50
5
5´
–30
Top strand

Conclusions

I. Maltose Operon.

1. Mal operon controlled by CRP & MalT (transcription factor)

2. CRP stimulates transcrption by shifting MalT from one set of binding sites to another (only 3 bp away)

3. Initial binding site of MalT is poorly aligned with (enhancing transcription from) the promoters

4. The “secondary” sites are better aligned with respect to the promoters and hence can facilitate transcription.