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# MalT, the regulatory protein of the *Escherichia coli* maltose system, is an ATP-dependent transcriptional activator

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**We show that MalT, the transcriptional activator of the *Escherichia coli* maltose regulon, specifically binds ATP and dATP with a high affinity ($K_d$ = 0.4 $\mu$M) and exhibits a weak ATPase activity. Using an abortive initiation assay, we further show that activation of open complex formation by MalT depends on the presence of ATP in addition to that of maltotriose, the inducer of the maltose system. Similar experiments in which ATP was replaced by ADP or AMP-PNP, a non-hydrolysable analogue of ATP, demonstrate that this reaction does not require ATP hydrolysis. As revealed by DNase I footprinting, both ATP and maltotriose are required for the binding of the MalT protein to the *mal* promoter DNA.**

*Key words:* ATP/*Escherichia coli*/maltose/transcriptional activator

## Introduction

The expression of the *Escherichia coli* maltose regulon, which comprises several operons encoding proteins involved in the uptake and catabolism of maltodextrins, is controlled by the product of the *malT* gene, a transcriptional activator, and by the presence of maltodextrins in the medium (reviewed by Schwartz, 1987). The purification of MalT in an active form and the development of a purified *in vitro* transcription system recently made the process of transcription activation by MalT amenable to biochemical analysis (Richet and Raibaud, 1987). MalT is a large protein ($M_r \sim 102\,000$) which is monomeric in solution and which binds at least three different ligands. It specifically binds DNA at different sites in the promoters whose activity it controls, recognizing a short, asymmetric motif called the MalT box (5'-GGAT/GGA-3') (Raibaud *et al.*, 1989). It binds maltotriose, the only maltodextrin able to act as a positive effector of MalT for activation of transcription *in vitro* (Raibaud and Richet, 1987). Finally, MalT is an ATP-binding protein, as suggested by the following lines of evidence: (i) ATP protects MalT against thermal denaturation, (ii) ATP can be photo-crosslinked to the protein, and (iii) the MalT polypeptide contains at its N-terminus an amino-acid sequence homologous to a conserved sequence (Gly-X-X-Gly-X-Gly-Lys-Thr-Thr) found in a large number of ATP- and GTP-binding proteins and considered to be part of the nucleotide binding domain (Richet and Raibaud, 1987). So far, the role of ATP as an effector of MalT is unknown.

In this paper, we further characterize the interaction of MalT with ATP and show that ATP-binding is essential for the activity of the protein. We also determine at which step in the transcription activation process each effector (ATP and maltotriose) is required.

## Results

### *MalT has a high affinity for ATP and dATP*

Previous studies have shown that MalT can bind ATP and dATP (Richet and Raibaud, 1987). To obtain more quantitative data on these interactions, we measured [$^3$H]ATP binding to MalT by two different techniques: equilibrium dialysis and ammonium sulphate precipitation of the nucleotide–MalT complex. Figure 1 shows Scatchard plot analysis of the data obtained in the presence of maltotriose and various concentrations of ATP. Both techniques indicate that, in the presence of maltotriose, MalT binds 0.5 molecules of ATP per monomer with a $K_d$ of 0.6 $\mu$M (equilibrium dialysis) or 0.4 $\mu$M (ammonium sulphate precipitation). Such an agreement between results obtained by the two methods was not expected since in two other cases (the CRP protein and the LacI repressor) the $K_d$ determined by equilibrium dialysis was 10-fold higher than the $K_d$ obtained by the ammonium sulphate precipitation (Anderson *et al.*, 1971; Jobe *et al.*, 1972). One likely explanation is that the retention effects (Silhavy *et al.*, 1975) and the slow kinetics of the interaction between MalT and ATP (see below) together result in the freezing of the equilibrium upon addition of ammonium sulphate at 0°C. Indeed, no [$^3$H]ATP-binding to the protein was detected when ammonium sulphate was added immediately after mixing ATP and MalT, and no loss of incorporated [$^3$H]ATP was observed when a large excess of unlabelled ATP was added together with the ammonium sulphate. At low concentrations of the nucleotide, the kinetics of the reaction are rather slow. For example, in the presence of 0.1 $\mu$M ATP, the equilibrium was reached only after 10 min at 20°C. The binding of ATP to MalT depends strictly on the presence of $Mg^{2+}$, as is usually observed for nucleotide-binding proteins. The omission of maltotriose also has a dramatic effect on ATP binding (Figure 1). In its absence, only 0.25 molecules of ATP were bound per monomer of MalT, with a $K_d$ of 0.7 $\mu$M (Figure 1B). The same results were obtained when maltose was substituted for maltotriose (Figure 1B), an observation which correlates very well with the strict specificity of MalT for maltotriose (Raibaud and Richet, 1987).

The affinity of MalT for other nucleotides was determined by measuring their competitive effect on [$^3$H]ATP binding to MalT using both techniques. These assays were performed in the presence of maltotriose. As shown in Table I, MalT binds dATP and AMP-PNP as effectively as ATP, has a relatively high affinity for ADP and GTP, but exhibits only

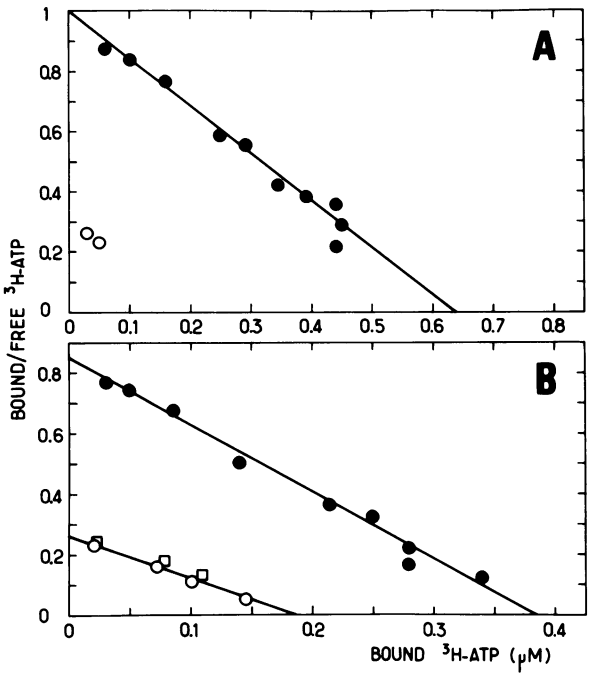


**Fig. 1.** ATP binding to the MalT protein. The data obtained by equilibrium dialysis (**A**) and by ammonium sulphate precipitation (**B**) are plotted by using the Scatchard representation. The assays were performed with 1.3 μM (A) or 0.8 μM MalT (B), in the presence of 1 mM maltotriose (●), 1 mM maltose (□) or in the absence of sugar (○). Most of the points represent the mean value of two independent measurements. The stoichiometry of nucleotide binding and the $K_d$ are calculated by making a slight correction to take into account the radiochemical purity of [$^3$H]ATP and the production of ADP from ATP.

**Table I.** Nucleotide specificity of the MalT protein

| Nucleotide | $K_d$ (μM) | | Activation of transcription initiation at *malPp* [b] (ApApC/promoter/min) |
|---|---|---|---|
| | Ammonium sulphate precipitation technique | Equilibrium dialysis | |
| none | – | – | ≤0.7 |
| ATP | 0.4 | 0.6 | 36 |
| ADP | 3 | 3 | 2 |
| AMP | $>10^3$ | $>10^3$ | ≤0.7 |
| dATP | 0.4 | – | 37 |
| AMP-PNP | ≤ 1[a] | – | 34 |
| GTP | 14 | 12 | – |
| CTP | 130 | 100 | ≤0.7 |
| UTP | 100 | 80 | ≤0.7 |

[a]The kinetics of AMP-PNP binding to MalT are much slower than with other nucleotides. After the 30 min incubation, the equilibrium was clearly not reached and, therefore, we can only give an upper limit for the $K_d$ of AMP-PNP.
[b]Abortive initiation assays were performed on *malPp* in the presence of 0.28 μM MalT and 10 μM of the indicated nucleotide as described in Materials and methods.

a weak affinity or no affinity at all for the other nucleotides tested. MalT therefore specifically binds the adenine nucleoside triphosphates, with no distinction being made between the ribo- and the deoxyribo-forms.

### *MalT has a weak intrinsic ATPase activity*

If ATP is used by MalT as an energy donor to drive some conformational change or as a $P_i$ donor to phosphorylate some compound, we might expect the protein to exhibit some uncoupled ATPase activity. The MalT protein, purified as previously described (Richet and Raibaud, 1987), showed a low ATPase activity which seemed to coelute with the protein during filtration through Bio-Gel A-0.5 m, the last step of the purification procedure (data not shown). This ATPase activity was stimulated 2- to 3-fold by maltotriose, strongly suggesting that this activity was indeed intrinsic to MalT. To investigate the possibility that the ATPase activity observed in the absence of maltotriose was due to a contaminating enzyme, we further purified MalT by anion-exchange chromatography. Figure 2A shows that both ATPase activities (maltotriose-dependent and maltotriose-independent) coeluted perfectly with the MalT protein. As already observed for ATP binding, no stimulatory effect was observed when maltose and maltotetraose, two maltodextrins which are not effectors of MalT for transcription activation, were substituted for maltotriose (Figure 2B and data not shown). The turn-over number of ATP hydrolysis was low, about 0.08 mol of ATP hydrolysed/min/mol of MalT (Figure 2A). Under the conditions used for the determination of the $K_d$ (Figure 1B), the hydrolysis was even lower (<0.01 mol of ATP/min/mol of MalT). The MalT protein hydrolyses dATP as effectively as ATP in the absence and in the presence of maltotriose (data not shown).

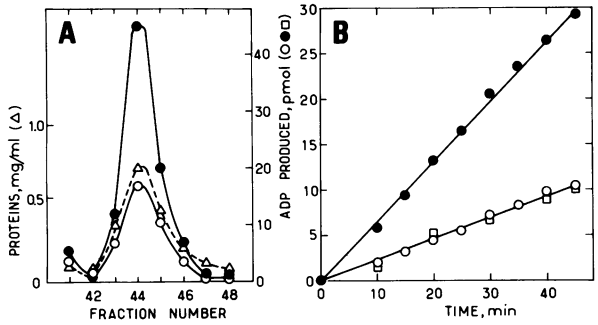


**Fig. 2.** MalT has an ATPase activity stimulated by maltotriose. **Panel A**: purified MalT protein [Fraction IV, ~90% pure (Richet and Raibaud, 1987)] was chromatographed on a mono-Q column as described in Materials and methods and the eluted fractions were assayed for protein content (△) and for ATPase activity. The ATPase assays were performed by incubating 2 μl of each fraction for 45 min at 30°C in the presence of 11 μM ATP, in the absence (○) or in the presence (●) of 1 mM maltotriose. **Panel B**: the time course of ATP hydrolysis by 0.58 μM MalT (fraction 44 of the mono-Q column) was determined at 6 μM ATP in the presence of 1 mM maltotriose (●), 1 mM maltose (□) or in the absence of sugar (○).

### *Abortive initiation at malPp, a new assay for MalT*

The ability of MalT to bind ATP suggested that ATP might be involved in the transcription activation process. However, the instability of the MalT protein under the standard temperature and salt conditions used for transcription assays and the partial protection conferred by ATP precluded a clear assessment of the role of ATP. We therefore searched for assay conditions under which the MalT protein would still be active at the end of the assay, even when ATP was omitted. We found that the replacement of the anion chloride by the anion citrate and incubation at 30°C instead of 37°C greatly improved the stability of MalT. For the transcription experiments reported here (as well as for the ATP-binding and the ATPase assays presented above), citrate salts have therefore been substituted for chloride salts.

None of the assays previously used (Zubay system and

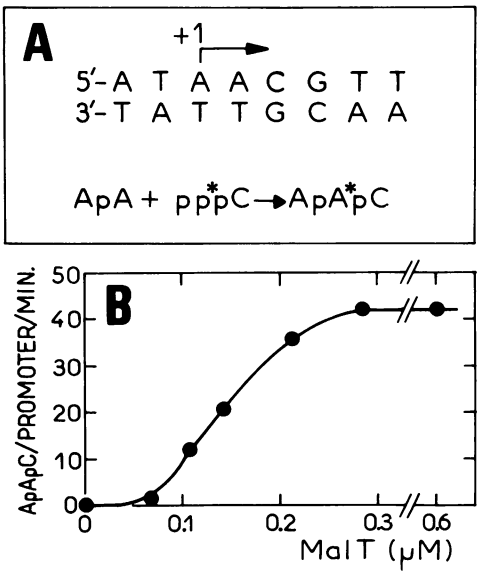


**Fig. 3.** Abortive initiation at *malPp*. **Panel A**: nucleotide sequence spanning the transcription start site (+1) of *malPp* and below, the reaction of abortive product synthesis at this promoter. **Panel B**: abortive initiation assays were carried out on *malPp*, in the presence of 1 mM maltotriose, 10 μM ATP and increasing concentrations of MalT, as described in Materials and methods.

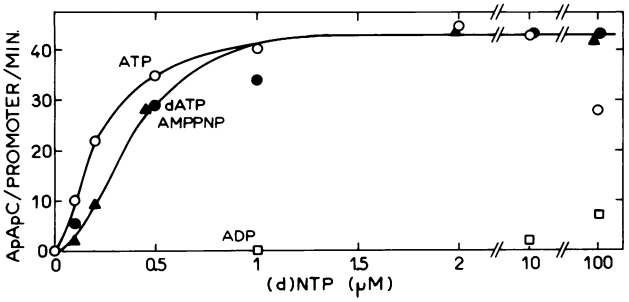


**Fig. 4.** Effect of different adenine nucleotides on activation of open complex formation by MalT. Abortive initiation assays were carried out on *malPp* in the presence of 0.28 μM MalT, 1 mM maltotriose and various concentrations of ATP (○), dATP (●), AMP-PNP (▲) or ADP (□), as described in Materials and methods.

run-off transcription) were adequate to examine the role of ATP since they could not be performed in its absence. Instead, we used the abortive initiation technique to monitor open complex formation (McClure, 1980). As shown in Figure 3A, abortive product synthesis at *malPp*, a promoter controlled by MalT (Raibaud *et al.*, 1985), depends only on the presence of ApA and CTP as substrates. When a DNA fragment carrying *malPp* was incubated in the presence of ApA, CTP and RNA polymerase, no ApApC synthesis was detected even after a 2-h incubation. However, when MalT, maltotriose and ATP were also present, trinucleotide synthesis was observed, the rate of which becomes constant only after several minutes following the addition of RNA polymerase (data not shown). This lag results from the slow rate of open complex formation (McClure, 1980). For a fixed concentration of RNA polymerase, this rate depends on the concentration of MalT, thereby providing an assay for MalT activity. In the experiments reported here, we used a fixed-time assay. Open complex formation was allowed to proceed for 10 min by incubating *malPp* with RNA polymerase, MalT and its effectors. The amount of open complexes formed was then quantified by adding ApA, [$\alpha$-$^{32}$P]CTP together with heparin (which traps the molecules of RNA polymerase not engaged in open complexes, thereby blocking their formation) and measuring the rate of ApApC synthesis. Under these conditions, the rate of trinucleotide synthesis was proportional to the amount of open complexes formed during the first incubation. Figure 3B shows the response to increasing concentrations of MalT in the presence of RNA polymerase (0.1 μM), ATP and maltotriose. The response curve is sigmoidal, as previously observed with the coupled transcription–translation system, and, as expected, open complex formation depends strictly on the presence of maltotriose (data not shown) (Raibaud and Richet, 1987; Richet and Raibaud, 1987).

### *ATP is required for activation of open complex formation by MalT*

Using the abortive initiation assay described above, we examined the effect of ATP on open complex formation in the presence of MalT. As shown in Figure 4, ApApC synthesis was ATP-dependent. The plateau was reached with 1 μM ATP, and half maximal activity of MalT was obtained with ~0.2 μM ATP, a value consistent with the $K_d$ measured above (Table I). No ApApC synthesis was detected in the absence of ATP at 0.28 μM MalT. When assayed in the absence of ATP, 1.1 μM of MalT gave the same signal as 0.07 μM MalT tested in the presence of a saturating concentration of ATP, indicating that ATP stimulates MalT activity at least 16-fold. No ApApC was synthesized when ATP was present only at the step of abortive product synthesis (data not shown), which indicates that, to be effective, ATP must be present during open complex formation. Controls showed that, even in the absence of ATP, MalT protein was still active at the end of the assay, thus ruling out a mere stabilizing effect of ATP (data not shown). Moreover, the possibility that the stimulatory effect of ATP reflected a stabilization of the open complex by an pppApA synthesized *in situ* from the ATP present was excluded by similar experiments performed with the *malEp* promoter. Although the *malEp* transcript begins with 5'-GpU, the activation of open complex formation also depended strictly on the presence of an adenine nucleoside triphosphate (data not shown). Altogether these results establish that ATP is required as a positive effector of MalT in the process of transcription activation.

Other nucleotides were examined for their ability to activate MalT. dATP was almost as effective as ATP (Figure 4). In contrast, ADP had only a weak stimulatory effect even when present at saturating concentrations (Figure 4). When assayed at 10 μM in the presence of 0.28 μM MalT, AMP, cAMP, dGTP, CTP, dCTP, UTP and TTP were totally inactive (Table I and data not shown). At saturating concentrations (100 μM), dGTP and GTP (which were tested on the *malEp* promoter) only weakly stimulated open complex formation (data not shown). These results are consistent with the specificity of MalT for the adenine nucleoside triphosphates observed in the nucleotide binding assays.

### *ATP hydrolysis is not required for activation of open complex formation*

As shown in Figures 4 and 5, AMP-PNP, a non-hydrolysable analogue of ATP, was as effective as ATP for activation

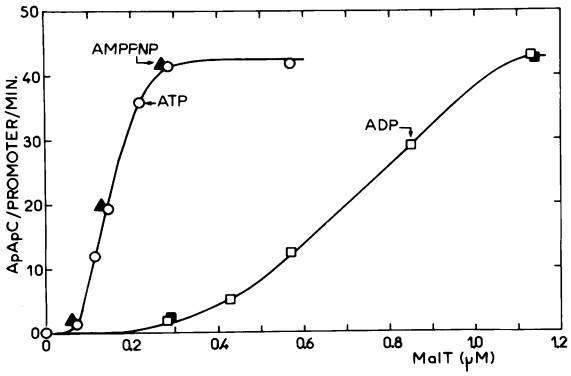


**Fig. 5.** MalT activity in the presence of ATP, AMP-PNP or ADP. Same experiment as that presented in Figure 3B, but performed in the presence of 10 μM ATP (○), 10 μM AMP-PNP (▲), 10 μM ADP (■) or 30 μM ADP (□).

of open complex formation by MalT. The conclusion that ATP hydrolysis is not needed at least up to formation of the open complex is further strengthened by the observation that the MalT-ADP form was fully competent for activation provided that MalT concentration was high enough. Indeed, although ADP had a small stimulatory effect when assayed at saturating concentrations in the presence of 0.28 μM MalT (see Figure 4), full activation could be achieved if the MalT concentration was raised to ~1 μM (Figure 5). The weak efficiency of ADP, apparent only at a low concentration of MalT, might therefore simply reflect a 5-times lower affinity of the MalT-ADP form for the *malPp* promoter as compared to the MalT-ATP or MalT-AMP-PNP forms.

### *MalT binding to the MalT box depends on the presence of both maltotriose and ATP*

We next examined whether both ATP and maltotriose, or only one of them, were required for the binding of MalT to its recognition sequences in the promoter, one of the first steps of the activation process. DNase I protection experiments were carried out with an end-labelled *malEp–malKp* DNA fragment in the presence of MalT and various combinations of effectors. Figure 6 shows that MalT binds to the MalT boxes only if ATP and maltotriose are present together. Neither the requirement for maltotriose nor the requirement for ATP reflect a stabilizing effect on MalT activity; the protein was still active at the end of the assay even if one effector had been omitted (data not shown). MalT binding to DNA shows the same specificity for maltodextrins and nucleotides as MalT activation of open complex formation. Binding was not detected when purified maltose or maltotetraose were substituted for maltotriose, and dATP and AMP-PNP could replace ATP whereas AMP, cAMP, (d)GTP, CTP and UTP could not (data not shown). It should be noted that, because protection shows a steep dependence upon MalT concentration (Raibaud *et al.*, 1989), a 4-fold effect of maltotriose or ATP on the affinity of MalT for the promoter DNA could account for the absence of protection observed when one effector was omitted.

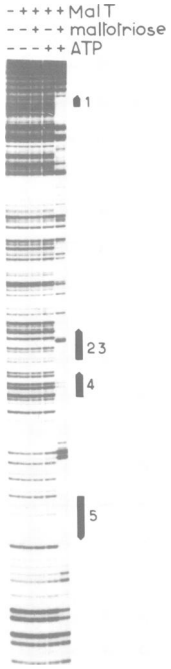


**Fig. 6.** MalT binding to *malEp–malKp*. DNase I protection experiments were carried out as described in Materials and methods. MalT (0.67 μM), maltotriose (1 mM) and ATP (0.1 mM) were present as indicated. The black arrowed boxes indicate the position of the different MalT recognition sites.

## Discussion

### *MalT, an ATP binding protein*

We have shown here that MalT, the transcriptional activator of the maltose regulon, specifically binds ATP and dATP with high affinity ($K_d$ = 0.4 μM). Surprisingly, the stoichiometry of the binding of ATP is 0.25 and 0.5 molecules of ATP per monomer of MalT in the absence and in the presence of maltotriose, respectively. One or more explanations could account for these observations. One trivial explanation is that the purified MalT protein is a mixture of MalT molecules either unable to bind ATP, able to bind ATP only in the presence of maltotriose, or able to bind ATP independently of the presence of maltotriose, which are present in the ratio 2:1:1, respectively. However, the fact that the same ratios were obtained with two different batches of purified MalT protein prepared >1 year apart does not support this hypothesis. Alternatively, a MalT monomer may possess two distinct ATP-binding sites, one of them binding ATP only in the presence of maltotriose. Although sequence comparisons revealed the existence of only one ATP-binding site homologous to that found in many ATP- and GTP-binding proteins (Walker *et al.*, 1982; Richet and Raibaud, 1987), the possibility remains that MalT contains an additional site that would be either degenerate or of another type (Husain *et al.*, 1986). However, to account for the fact that, in the presence of maltotriose, the ATP-binding sites appear as a homogeneous population with regard to their affinity for ATP and the other nucleotides tested, one has to assume that both types of sites have the same properties. Such a restriction renders the latter hypothesis less plausible.

Finally, an attractive possibility is that MalT in fact exists as an oligomer, e.g. a tetramer, in which nucleotide binding to one protomer prevents binding to the other protomers through anticooperative effects. Binding of maltotriose might promote ATP binding to a second subunit by relieving part of these effects. Such an anticooperative binding of ATP has recently been reported for two

oligomeric ATP-binding proteins, the Lon protease (Menon and Goldberg, 1987) and the Rho protein (Stitt, 1988). It may be argued that previous studies showed that MalT exists as a monomer (Richet and Raibaud, 1987). However, these experiments were done in the presence of chloride whereas the ATP-binding assays described here were performed in the presence of citrate, an anion known for stabilizing oligomeric structures by strengthening hydrophobic interactions (Raibaud and Goldberg, 1976; Arakawa and Timasheff, 1984).

### *Role of ATP in activation of transcription initiation by MalT*

The main conclusion of this work is that activation of transcription initiation by MalT requires, in addition to maltotriose, the presence of ATP (or dATP) as an effector of MalT. The fact that MalT needs to interact with two different ligands to achieve activation strongly suggests that the mechanism by which the protein acts is a complex one. As shown by DNase I footprinting, MalT binding to its recognition sites present in the *malEp–malKp* promoters depends on the presence of both ATP and maltotriose, which indicates that both of them are required at an early stage of the transcription initiation process. A simple model is that binding of both ATP and maltotriose drive MalT protein into a conformation appropriate for binding of a MalT box. However, it should be pointed out that MalT binding to the *malEp–malKp* region, which contains five MalT boxes, appears to be highly cooperative (Raibaud *et al.*, 1989). Therefore, one cannot exclude the alternative scenario in which one ligand affects the interaction of the MalT monomer with a MalT box whereas the binding of the other ligand promotes interactions between different MalT monomers, thereby stabilizing the nucleoprotein complex.

The finding that ATP is involved in transcription activation by MalT raises several questions about its role. Does ATP hydrolysis occur at one step of the activation process, as suggested by the ability of MalT to hydrolyse ATP? If so, what is the nature of the event triggered by the hydrolysis of ATP? As clearly shown here, activation of open complex formation by MalT does not require ATP hydrolysis. ADP and AMP-PNP, a non-hydrolysable analogue of ATP, can fully replace ATP for activation of open complex formation. Thus, we infer that, at least up to this step, ATP merely acts as an allosteric effector, i.e. that neither the potential energy of ATP hydrolysis nor the phosphorylation of MalT or maltotriose are needed. However, open complex formation represents only one part of the transcription initiation process. Completion of the reaction requires that the RNA polymerase leave the promoter, i.e. the open complex be converted into an elongating complex (McClure, 1985). The possibility therefore remains that ATP hydrolysis is needed for promoter clearance, or even later on, prior to the next reinitiation event in the recycling of the promoter. Any of these steps might conceivably involve dissociation of the nucleoprotein complex formed by MalT at the promoter. In this regard, the observation that the MalT-ADP form seems to have a lower affinity for promoter DNA than the MalT-ATP form is provocative.

Alternatively, ATP could be a mere allosteric effector endowed with a regulatory role, for example allowing coupling between the catabolism of maltodextrins and the energy charge of the bacterium. This seems rather unlikely since the $K_d$ value of MalT for ATP is 3–4 orders of magnitude lower than the intracellular concentration of ATP (Chapman and Atkinson, 1973). Moreover, the ratio of the $K_d$ for ADP to the $K_d$ for ATP (~7) does not seem appropriate for sensing the energy charge of the cell [under normal conditions, the intracellular concentration of ATP is ~ 6-fold higher than the concentration of ADP (Bagnara and Finch, 1973)].

To our knowledge, MalT is the first ATP-dependent transcriptional activator so far described in prokaryotes. This requirement for ATP is a property which, together with other features of the MalT protein (its size and the asymmetry of its recognition sequence) distinguishes it from the classical bacterial regulators such as the CI or the CRP proteins. It is therefore tempting to speculate that MalT might activate transcription initiation by a mechanism different from that used by CI or CRP to activate $P_R$ or *lacZp* (de Crombrugghe *et al.*, 1984; Ptashne, 1986). Interestingly, in many respects, MalT rather resembles the DnaA protein, the activator of initiation of DNA replication in *E.coli*. Both are high-affinity ATP-binding proteins which upon binding to their recognition sequences at *malEp–malKp* and at *oriC*, respectively, form similar nucleoprotein complexes (Fuller *et al.*, 1984; Sekimizu *et al.*, 1987; Raibaud *et al.*, 1989). As recently shown by Bramhill and Kornberg (1988), DnaA protein acts by opening the DNA at an adjacent region in *oriC*, a process requiring the presence of ATP. It will be interesting to see whether the similarities between MalT and DnaA protein have a functional significance and in particular whether MalT facilitates open complex formation at the *mal* promoters by locally untwisting the DNA.

## Materials and methods

### *Reagents and buffer*

AMP-PNP and ADP were purchased from Boehringer; ADP contained <0.2% ATP as specified by the manufacturer. [2,5′,8-$^3$H]ATP (51 Ci/mmol) was obtained from Amersham. It was about 95% radiochemically pure as tested by TLC on PEI cellulose. Maltotriose was from Sigma. Maltose (from Pfanstiehl) and maltotetraose (from Boehringer) were purified from traces of contaminating maltotriose as previously described (Raibaud and Richet, 1987). Buffer C contains 40 mM Hepes–KOH (pH 8.0 at 20°C), 33 mM tri-potassium citrate, 10 mM magnesium acetate and 0.1 mM EDTA.

### *DNA fragments*

The 312 bp *PstI–EcoRI* fragment containing *malPp* and the 457 bp *EcoRI–BamHI* fragment containing the divergent *malEp* and *malKp* promoters were prepared from pOM19 and pOM18, respectively, as previously described (Richet and Raibaud, 1987). DNA fragment concentrations were determined by measuring the enhancement of fluorescence of *bis*-benzimidazole (Hoechst 33258) observed upon binding of the dye to DNA, with a mini-fluorometer (TKO 100, Hoefer Scientific Instruments).

### *Proteins*

MalT was purified in the presence of ATP as previously described (Richet and Raibaud, 1987). MalT preparations devoid of ATP were obtained as follows. Purified MalT (~0.7 mg) was precipitated by adding 2 vols of 3 M ammonium sulphate in 50 mM Tris–HCl (pH 7.7), 0.1 M KCl, 0.1 mM EDTA and 10% sucrose. After washing with the same solution, the pellet was dissolved in 40 μl containing 50 mM Tris–HCl (pH 7.7), 0.1 M tri-potassium citrate, 0.1 mM EDTA and 10% sucrose, and filtered on a 2.5 ml Sephadex G-25 column equilibrated in the same buffer. For the MalT preparations to be used in DNase I protection experiments, the citrate salt was replaced by 0.3 M KCl. The ATP-free MalT preparations could be stored for several months at −20°C without any loss of activity.

Protein concentrations were determined by the procedure of Lowry *et al.* (1951) using crystallized BSA (Sigma) as a standard and taking $E^{1\%}_{280nm} = 6$

for the BSA. The molar concentration of MalT was obtained by determining the amino acid content of a sample of purified MalT protein with a Beckman 7300 amino acid analyzer and by dividing the molar concentration of a given amino acid by the number of residues of that amino acid per monomer as deduced from the sequence of the *malT* gene (Cole and Raibaud, 1986). The final value represents the average of the values obtained with 11 different amino acids. A purified MalT fraction containing 1 mg/ml protein, as determined by the Lowry method, corresponds to 8 ± 0.8 μM MalT.

Purified RNA polymerase holoenzyme (60% active) and CRP were kindly provided by the laboratory of H.Buc (Institut Pasteur).

#### FPLC of MalT on a mono-Q-column

Purified MalT (0.7 mg in a buffer containing 50 mM Tris−HCl (pH 7.7), 10 μM ATP, 0.1 mM EDTA, 1 mM $MgCl_2$, 10% sucrose and 0.05 M KCl) was applied to a 1 ml mono-Q column (Pharmacia) equilibrated with the same buffer. After washing with 5 ml of equilibration buffer, elution was carried out with a 10 ml linear gradient from 0.05 to 0.6 M KCl. The flow rate was kept at 0.5 ml/min throughout the procedure and 0.25 ml fractions were collected. The MalT protein eluted at ~0.3 M KCl.

#### ATP-binding assays

Equilibrium dialysis were performed at 5°C as follows: 0.5 ml of buffer C containing 0.5 μCi [$^3$H]ATP, various concentrations of unlabelled ATP (0.05−2 μM) and, when indicated, 1 mM maltotriose, were pipetted into one well of a 24-well tissue culture treated plate (Nunc, Denmark). A small filter (VSWP1300, Millipore) was laid at the surface of the solution, with the glossy face up. Ten minutes later, 26 μl of the same solution mixed to 4 μl of a 10 μM ATP-free MalT fraction were pipetted onto the filter. The well was sealed with Parafilm. At different times, 4 μl of the protein solution were removed and diluted into 0.5 ml of water, before mixing with 5 ml of liquid scintillation cocktail (Ready gel, Beckman) for counting. In all of the experiments, a stationary state was observed between 4.5 and 6.5 h after the start. The amount of ATP bound to MalT was calculated by using the mean value of the 4.5 and 5.5 h samplings.

Ammonium sulphate precipitation assays were carried out essentially as described by Anderson *et al.* (1971). A 2.5 μl aliquot of a 10 μM ATP-free MalT fraction was added to 27.5 μl of buffer C containing 0.33 mg/ml BSA, 1 μCi/ml [$^3$H]ATP and various concentrations of unlabelled ATP (0.05−3 μM), and the mixture was incubated for 15 min at 20°C. The tube was then chilled on ice for 2 min and the proteins precipitated by adding 90 μl of buffer C containing 3.1 M ammonium sulphate (when the binding assay was done in the presence of maltose or maltotriose, the sugar was also included in the ammonium sulphate solution). After 5 min at 0°C, the precipitate was collected by a 10 min centrifugation at 5°C in an Eppendorf centrifuge, dissolved in 0.5 ml of water and counted as above. Control experiments show that (i) at the end of the experiments ~25 and 10% of the ATP present in the mixture have been hydrolysed to ADP in the equilibrium dialysis and ammonium sulphate precipitation experiments, respectively, and (ii) no loss of MalT activity was detected after a 30 min incubation at 20°C or a 5 h incubation at 5°C. The affinity of MalT for other nucleotides was determined by performing competition assays in which MalT was incubated in the presence of 1 mM maltotriose, 0.1 μM [$^3$H]ATP and various concentrations of competing nucleotide. For the ammonium sulphate precipitation assay, a 30 min incubation at 20°C was required to reach the equilibrium.

#### ATPase activity assays

The assays were carried out by incubating MalT at 30°C in 20 μl of buffer C containing 30 mM KCl, 1 mM dithiothreitol, 100 μg/ml acetylated BSA, 0.2 μCi [$^3$H]ATP (or [$\alpha$-$^{32}$P] dATP) and the indicated concentrations of unlabelled ATP (or dATP). At varying times, 2 μl of the mixture were spotted on thin-layer PEI cellulose plates (Schleicher and Schuell) prespotted with 1 μl containing 20 mM each EDTA, ATP and ADP. ADP was separated from ATP by ascending chromatography in 0.5 M LiCl and 1 M formic acid. The ADP and ATP spots were cut out and counted in 5 ml of scintillation liquid.

#### Abortive initiation assays

Abortive initiation assays were performed essentially as described by McClure (1980). ATP-free MalT was first preincubated at 20°C in 16 μl of buffer C containing 100 μg/ml acetylated BSA, 1 mM dithiothreitol, 1 mM maltotriose and various concentrations of (d)NTP. After ~15 min, 2 μl of a *malPp* DNA fragment solution (75 nM) and 2 μl of a RNA polymerase holoenzyme solution (1 μM) were added and the mixture incubated for 10 min at 30°C. Abortive initiation was started by the addition of 2 μl containing 5 mM ApA, 0.5 mM [$\alpha$-$^{32}$P]CTP (~0.4 Ci/mmol) and 500 μg/ml heparin, and allowed to proceed for 15 min at 30°C. The reaction product (ApApC) was separated from free [$\alpha$-$^{32}$P]CTP by chromatography and quantified by cutting out and counting, as described by McClure (1980). We verified that ApApC synthesis was linear over at least 20 min after the initiation of the reaction. We also verified that preincubation of MalT with DNA was not necessary. With this system, the effect of any (d)NTP on MalT activity could be assayed, except for GTP which is the next nucleotide to be incorporated after CTP. GTP was assayed by using the *malEp* promoter which directs the abortive synthesis of GpUpUpU. The assay was carried out as above with the DNA fragment containing *malEp* and *malKp*, except that 0.2 mM cAMP and 30 nM CRP were included in the reaction mixture (Richet and Raibaud, 1987) and that ApA and [$\alpha$-$^{32}$P]CTP were replaced by GpU and [$\alpha$-$^{32}$P]UTP.

#### DNase I protection experiments

The *Eco*RI−*Bam*HI containing *malEp* and *malKp* was labelled at the *Bam*HI end by filling in with the DNA polymerase I Klenow fragment in the presence of [$\alpha$-$^{32}$P]dGTP and dATP, followed by ethanol precipitation. The 20 μl reaction mixture contained 40 mM Tris−HCl (pH 8.0), 40 mM KCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mM EDTA, 1 mM DTT, 100 μg/ml acetylated BSA, ~1 nM end-labelled DNA fragment, 0.67 μM ATP-free MalT and, when indicated, 1 mM maltotriose and 0.1 mM ATP. After a 10 min preincubation at 25°C, 2 μl of 1 μg/ml DNase I [in 40 mM Tris−HCl (pH 8.0), 40 mM KCl, 1 mM $CaCl_2$] were added and the reaction mixture further incubated for 1 min at 25°C. The digestion was stopped by adding 20 μl of a solution containing 0.6 M sodium acetate, 50 mM EDTA and 20 μg/ml sonicated plasmid DNA. The DNA was recovered by ethanol precipitation and resuspended in 4 μl of 94% formamide, 10 mM EDTA (pH 7.5), 0.1% (w/v) xylene cyanol and 0.1% (w/v) bromophenol blue. The samples were electrophoresed through an 8% (w/v) polyacrylamide sequencing gel (0.3 mm thick) in the presence of 8.3 M urea. The gel was fixed, dried and autoradiographed on Kodak XAR-5 film without a screen.

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