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Manganese transport and its regulation in bacteria

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Abstract

Regulation of manganese acquisition by bacteria occurs by both biochemical regulation of the activity of the transporters and transcriptional regulation of gene expression. Structural analysis suggests that calcium ions may regulate the function of an Mn ATP-binding cassette (ABC)-permease in *Synechocystis* 6803, a cyanobacterium, as well as in a number of other bacteria. The expression of genes encoding the manganese transporter in *Synechocystis* 6803 is regulated by a two-component signal-transduction mechanism that has not been previously observed for manganese and zinc transport in bacteria.

Introduction

Manganese is an essential trace element in almost all organisms. During oxygenic photosynthesis, Mn plays an essential role in the oxidation of water. We have previously identified and characterized an ATP-binding cassette (ABC)-permease complex which has a central role in the acquisition of Mn by the cyanobacterium *Synechocystis* 6803 [1,2]. This Mn ABC-permease consists of MntC, an extracellular solute-binding protein (SBP), MntB, a membrane-spanning protein, and MntA, an intracellular ABC protein. BLAST analysis with MntC as the probe identified a set of SBPs in a variety of bacteria. The corresponding transporters have been implicated in the transport of metal ions, primarily Mn and Zn [3,4].

Form of MntC

The protein sequences of five SBPs of ABC-permeases were compared using ClustalW alignment (Figure 1). MntC in *Synechocystis* 6803 [1] and PsaA in *Streptococcus pneumoniae* [5] are SBPs in Mn ABC-permeases, whereas TroA in *Treponema pallidum* [6] and ZnuA in *Escherichia coli* [7] are SBPs in Zn ABC-permeases. Figure 1 also includes an uncharacterized SBP in the Gram-negative bacterium *Haemophilus influenzae*. This protein is a close homologue of YfeA, the SBP component of an Fe- and Mn-uptake system in *Yersinia pestis* [8]. Residues corresponding to His-89, His-154 and Asp-295 in MntC are fully conserved in all five SBPs (Figure 1). All five of the

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Key words: ATP-binding cassette-permease, MntC, *Synechocystis* 6803.

Abbreviations used: ABC, ATP-binding cassette; SBP, solute-binding protein.

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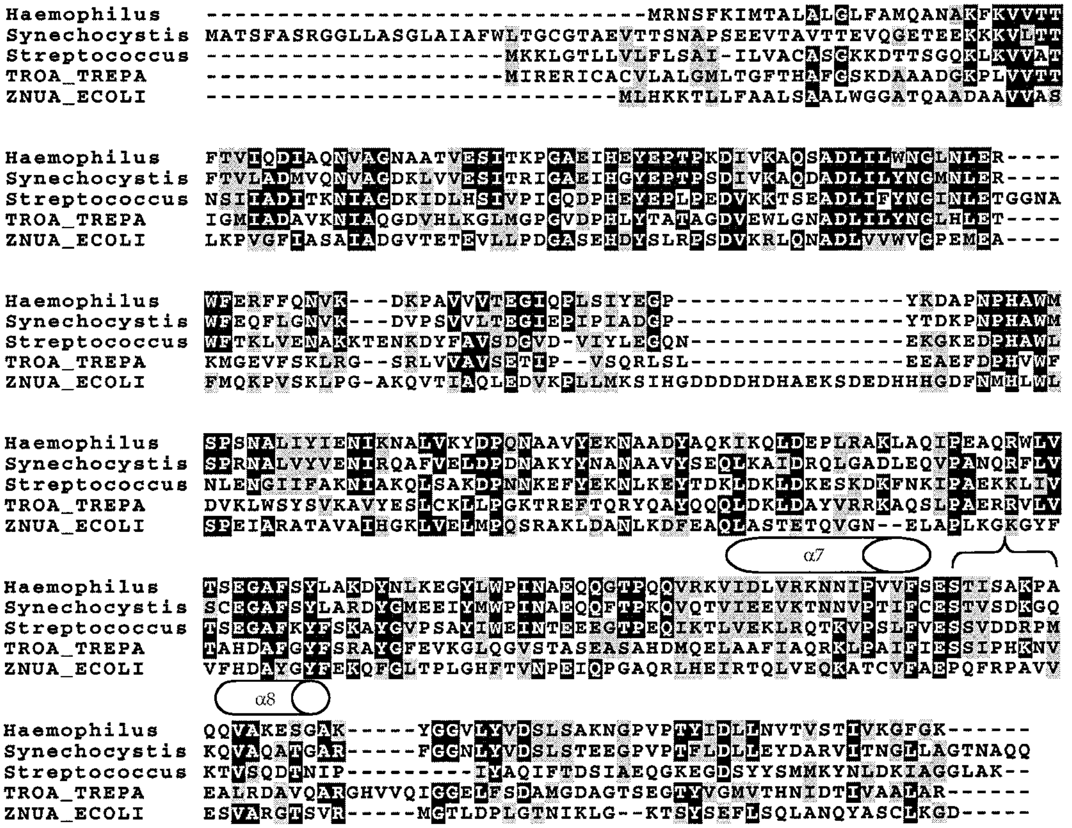
Figure 1

Sequence comparison of the proteins YfeA (*Haemophilus*), MntC (*Synechocystis*), PsaA (*Streptococcus*), TroA (TROA_TREPA) and ZnuA (ZNUA_ECOLI), a model of MntC and a phylogenetic tree

(Top panel) The areas of similarity and identity are indicated with grey and black shading, respectively. Three specific regions are additionally highlighted. α-Helices 7 and 8 are indicated by the cylinders shown above the sequence. The EF-hand domain is indicated by a horizontal bracket above the sequence of interest. (Bottom left panel) The model of MntC was generated using the homology module of the Accelyrs InsightII suite program (v2000). The α-helical and β-sheet regions are shown using a ribbon diagram. (Bottom right panel) The phylogenetic tree was generated by ClustalW analysis of the sequences compared in the top section of the figure.



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Haemophilus     -------------------------------MRNSFKIMTALALGLFAMQANAKFKVVTT
Synechocystis   MATSFASRGGLLASGLAIAFWLTGCGTAEVTTSNAPSEEVTAVTTEVQGETEEKKKVLTT
Streptococcus   ---------------------MKKLGTLLVLFLSAI-ILVACASGKKDTTSGQKLKVVAT
TROA_TREPA      ---------------------MIRERICACVLALGMLTGFTHAFGSKDAAADGKPLVVTT
ZNUA_ECOLI      -----------------------------MLHKKTLLFAALSAALWGGATQAADAAVVAS

Haemophilus     FTVIQDIAQNVAGNAATVESITKPGAEIHEYEPTPKDIVKAQSADLILWNGLNLER----
Synechocystis   FTVLADMVQNVAGDKLVVESITRIGAEIHGYEPTPSDIVKAQDADLILYNGMNLER----
Streptococcus   NSIIADITKNIAGDKIDLHSIVPIGQDPHEYEPLPEDVKKTSEADLIFYNGINLETGGNA
TROA_TREPA      IGMIADAVKNIAQGDVHLKGLMGPGVDPHLYTATAGDVEWLGNADLILYNGLHLET----
ZNUA_ECOLI      LKPVGFIASAIADGVTETEVLLPDGASEHDYSLRPSDVKRLQNADLVVWVGPEMEA----

Haemophilus     WFERFFQNVK---DKPAVVVTEGIQPLSIYEGP----------------YKDAPNPHAWM
Synechocystis   WFEQFLGNVK---DVPSVVLTEGIEPIPIADGP----------------YTDKPNPHAWM
Streptococcus   WFTKLVENAKKTENKDYFAVSDGVD-VIYLEGQN---------------EKGKEDPHAWL
TROA_TREPA      KMGEVFSKLRG--SRLVVAVSETIP--VSQRLSL---------------EEAEFDPHVWF
ZNUA_ECOLI      FMQKPVSKLPG-AKQVTIAQLEDVKPLLMKSIHGDDDDHDHAEKSDEDHHHGDFNMHLWL

Haemophilus     SPSNALIYIENIKNALVKYDPQNAAVYEKNAADYAQKIKQLDEPLRAKLAQIPEAQRWLV
Synechocystis   SPRNALVYVENIRQAFVELDPDNAKYYNANAAVYSEQLKAIDRQLGADLEQVPANQRFLV
Streptococcus   NLENGIIFAKNIAKQLSAKDPNNKEFYEKNLKEYTDKLDKLDKESKDKFNKIPAEKKLIV
TROA_TREPA      DVKLWSYSVKAVYESLCKLLPGKTREFTQRYQAYQQQLDKLDAYVRRKAQSLPAERRVLV
ZNUA_ECOLI      SPEIARATAVAIHGKLVELMPQSRAKLDANLKDFEAQLASTETQVGN--ELAPLKGKGYF
                                                      α7

Haemophilus     TSEGAFSYLAKDYNLKEGYLWPINAEQQGTPQQVRKVIDLVRKNNIPVVFSESTISAKPA
Synechocystis   SCEGAFSYLARDYGMEEIYMWPINAEQQFTPKQVQTVIEEVKTNNVPTIFCESTVSDKGQ
Streptococcus   TSEGAFKYFSKAYGVPSAYIWEINTEEEGTPEQIKTLVEKLRQTKVPSLFVESSVDDRPM
TROA_TREPA      TAHDAFGYFSRAYGFEVKGLQGVSTASEASAHDMQELAAFIAQRKLPAIFIESSIPHKNV
ZNUA_ECOLI      VFHDAYGYFEKQFGLTPLGHFTVNPEIQPGAQRLHEIRTQLVEQKATCVFAEPQFRPAVV
                  α8

Haemophilus     QQVAKESGAK-----YGGVLYVDSLSAKNGPVPTYIDLLNVTVSTIVKGFGK------
Synechocystis   KQVAQATGAR-----FGGNLYVDSLSTEEGPVPTFLDLLEYDARVITNGLLAGTNAQQ
Streptococcus   KTVSQDTNIP---------IYAQIFTDSIAEQGKEGDSYYSMMKYNLDKIAGGLAK--
TROA_TREPA      EALRDAVQARGHVVQIGGELFSDAMGDAGTSEGTYVGMVTHNIDTIVAALAR------
ZNUA_ECOLI      ESVARGTSVR-----MGTLDPLGTNIKLG--KTSYSEFLSQLANQYASCLKGD-----
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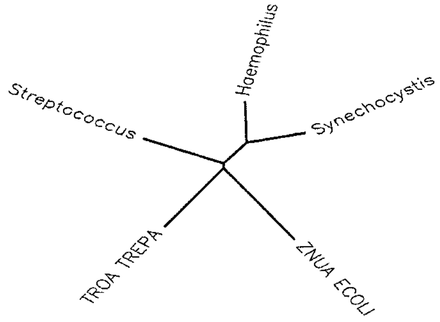

SBPs shown follow predicted paradigms for metal-co-ordinating amino acids [3]. The metal-co-ordinating residue Glu-220 in MntC is replaced by His in TroA and ZnuA.

Sequence comparison indicates that the overall homology between PsaA and MntC is about 30 %. PsaA is similar to both MntC and YfeA proteins. However, the *Haemophilus* YfeA and the *Synechocystis* MntC proteins share greater similarity ($\approx 57\,\%$). The two Zn permease SBPs included in our analysis, TroA and ZnuA, are only about 17 % similar to MntC, PsaA or YfeA. TroA and ZnuA are also about 20 % similar to each other. A phylogenetic tree based on these analyses (Figure 1) indicates that *Haemophilus* YfeA is the closest homologue to *Synechocystis* MntC, and we propose that this protein is a component of an Mn ABC-permease.

In addition to the metal-binding residues, there are other areas that are highly conserved among these SBPs (Figure 1). In general the conservation is highest in regions predicted to be α-helices. A homology model of MntC (Figure 1) was built using the X-ray crystal structure of PsaA [9] as the structural basis. The model shows that although only 30 % similar at the primary sequence level, the predicted structure of MntC is very similar to the structure of PsaA. The model indicates that a loop region between helices $\alpha7$ and $\alpha8$ (Figure 1) in MntC resembles an EF-hand domain. This region of the MntC protein (between residues Glu-269 and Glu-274) is predicted to ligate Ca^{2+} via the carboxylate oxygens of glutamate and aspartic acid residues. Corresponding residues are present in PsaA, but not in YfeA, TroA or ZnuA proteins. We have previously determined that Mn transport by MntABC can be enhanced by Ca^{2+} [2]. We have also determined that Mn binding to MntC *in vitro* is enhanced by Ca^{2+}. We predict that the function of PsaA will be similarly modulated by Ca^{2+} and that Ca^{2+} probably does not affect TroA-, ZnuA- or YfeA-related transport.

Transcriptional regulation

The Mn ABC-permease complex in *Synechocystis* 6803 functions when the external Mn concentration is sub-micromolar. The complex is under strict transcriptional regulation. Recently we have identified a two-component regulatory system that controls expression of the *mnt* operon. This sensor and regulator gene pair in *Synechocystis* 6803 is novel in that this mode of regulation is distinctly different from the known transcriptional regulators that control Mn-uptake systems in other bacteria. Two-component signal-transduction control systems have so far been identified for copper and silver in *E. coli* and *Pseudomonas* spp. [10].

Several transcriptional regulators for bacterial Mn homoeostasis have been identified. Que and Helmann [11] have determined that MntR, a DtxR diphtheria toxin-repressor protein homologue, regulates Mn uptake in *Bacillus subtilis* cells. ScaR, a homologue of MntR in *Streptococcus gordonii*, acts as a repressor for the *sca* operon that encodes an Mn-permease similar to the MntABC transporter [12]. In *E. coli* cells, Fur, an iron-dependent regulator, and MntR, a manganese-dependent regulator, control expression of the *mntH* Mn-transporter gene [13]. None of these organisms has any known two-component signal-transduction system for Mn.

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