Crystal Structure of MgtE Mg^{2+} Transporter

Magnesium ion, Mg^{2+}, is one of the most abundant divalent cations in biological systems and vital for all living organisms. For example, Mg^{2+} is essential for ATP utilization and is a cofactor for myriad enzymes, e.g., those in ribosomes. The chemical properties of Mg^{2+} are quite unique among biological cations. Its ionic radius is the smallest whereas its hydrated radius is by far the largest among all cations. Therefore, it has been a mystery how Mg^{2+} transporting proteins selectively recognize and dehydrate the very large fully-hydrated Mg^{2+} cation for its transport.

The MgtE family of Mg^{2+} transporters is ubiquitously distributed in all phylogenetic domains, and human homologues have been functionally characterized and suggested to be involved in magnesium homeostasis. However, its Mg^{2+} transporting mechanism is absolutely unclarified.

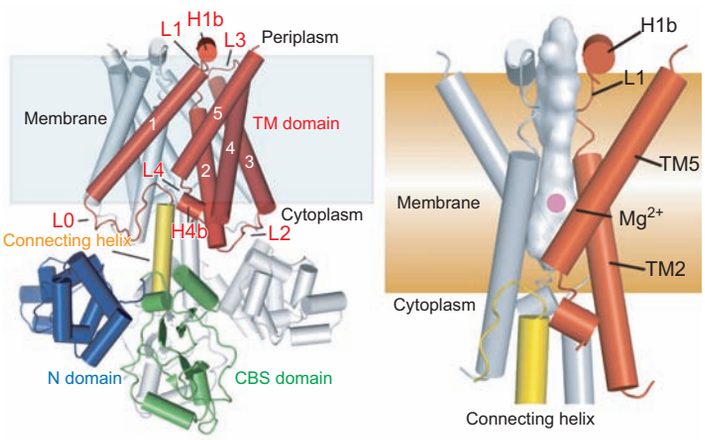

Fig. 1. Structure of MgtE Mg^{2+} transporter. **(a)** The MgtE dimer is viewed in the plane of the membrane, highlighting the N domain (blue), CBS domain (green), connecting helix (yellow), and transmembrane (TM) domain (red) in one subunit. The other subunit is in grey. The transmembrane helices of one subunit are numbered. The membrane surface is indicated. **(b)** Solvent-accessible surface of pore with pore-forming transmembrane helices. The putative Mg^{2+} is shown in purple.

To understand the transport mechanism by MgtE, we determined the crystal structure of full-length *Thermus thermophilus* MgtE at 3.5 Å resolution using beamline **BL41XU** (Fig. 1(a)). The transporter adopts a homodimeric architecture, consisting of the carboxy-terminal transmembrane (TM) domains with five TM helices and the amino-terminal cytosolic domains, which are composed of the superhelical N domain and tandemly repeated cystathionine-β-synthase (CBS) domains. The linker region between the cytosolic and TM domains contains a stretching helix referred to as a 'connecting helix' which is oriented perpendicularly to the membrane interface.

The MgtE structure reveals a putative continuous ion-conducting pathway, formed mainly by the TM2 and TM5 helices (Fig. 1(b)), which does not traverse the membrane at the cytosolic side. The TM5 helices from both subunits close the pore through interactions

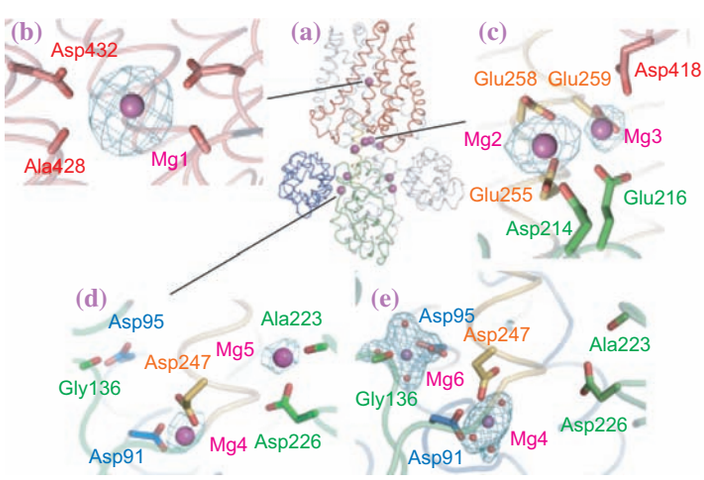

Fig. 2. Putative Mg^{2+} binding sites. The coloring scheme is the same as in Fig. 1. **(a)** Side view of overall structure with bound Mg^{2+}. **(b - d)** Close-up view of respective Mg^{2+} (Mg1-5) binding sites in full-length MgtE structure, with F_o-F_c simulated annealing omit map (contoured at 4.0 σ) calculated with full-length structure excluding Mg^{2+}. **(e)** Close-up view of Mg4 and Mg6 in cytosolic domain with F_o-F_c simulated annealing omit map (contoured at 4.0 σ) calculated with cytosolic domain structure excluding Mg^{2+} and water molecules.

with 'connecting helices'. Therefore, the current structure seems to represent a closed state for ion conduction.

We identified five strong residual electron density peaks (4.0 σ) per MgtE monomer in the full-length MgtE structure (Fig. 2). Considering that the crystallization condition included 40 mM magnesium acetate, and that all of the electron densities are close to the conserved acidic residues, it is reasonable to interpret the densities as Mg^{2+} ions, which are referred to as Mg1-5.

Mg1 is bound to the strictly conserved Asp 432 within the pore, which is presumably critical for the Mg^{2+} transporting activity. The other four putative Mg^{2+} ions (Mg2-5) are bound at the interface between the connecting helices and the other domains, and this may lock the current closed conformation of the pore.

We also determined the cytosolic domain structures in the presence and absence of Mg^{2+} at 2.3 Å and 3.9 Å resolutions, respectively. A structural comparison of the cytosolic domains in the presence and absence of Mg^{2+} revealed that the respective structures of the N and CBS domains are essentially identical, but the domain organization is markedly changed (Fig. 3(a,b)). In particular, the structures of the dimeric CBS domains are significantly changed. On the basis of the structural comparison of the cytosolic domains in two states, we propose here the following transport mechanism. In the presence of Mg^{2+}, the CBS domains tightly dimerize and the following connecting helices are fixed by Mg2-, Mg3- and Mg4-mediated interactions with the cytosolic and TM domains, which close the ion-conducting pore and lock the closed state (Fig. 3(c)). In contrast, in the absence of Mg^{2+}, the dimer interface of the CBS domains is loosened; consequently, the connecting helices are 'unlocked' and rotated by 20° to swing away from each other (Fig. 3(b)). This movement of the connecting helices disrupts the interactions between the connecting helices and the TM domains, thus allowing the rearrangement of the pore-forming TM helices (TM2 and TM5) and leading to the opening of the ion-conducting pore (Fig. 3(d)). Altogether, the cytosolic domain of MgtE may function as a 'Mg^{2+} sensor', which regulates the gating of the Mg^{2+} transporting pore by sensing the intracellular Mg^{2+} concentration, representing a putative negative feedback or Mg^{2+} homeostasis mechanism (Fig. 3(c,d)).

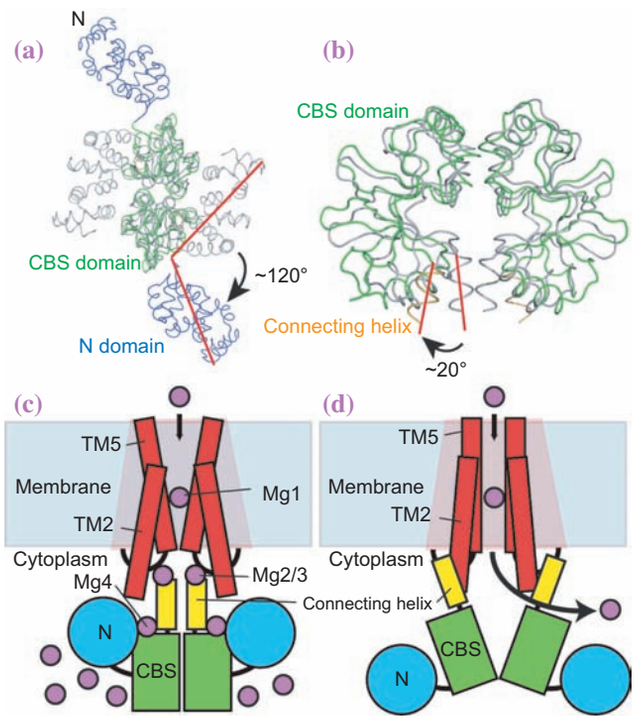

Fig. 3. Proposed Mg^{2+} homeostasis mechanism. **(a, b)** Structural comparison of Mg^{2+}-bound and Mg^{2+}-free cytosolic domains, which are superimposed on CBS domains and viewed from cytoplasm **(a)** and in membrane plane **(b)**. The coloring scheme of the Mg^{2+}-free cytosolic domain is the same as that in Fig. 1. The Mg^{2+}-bound cytosolic domain is in grey. **(c, d)** Proposed gating mechanism. Closed state at high intracellular Mg^{2+} concentration **(c)**. Open state at low intracellular Mg^{2+} concentration **(d)**.

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References

[1] *M. Hattori, Y. Tanaka, S. Fukai, R. Ishitani, O. Nureki: Nature* ***448*** *(2007) 1072.*
[2] M. Hattori *et al.*: Acta Crystallogr. F **63** (2007) 682
[3] Y. Tanaka *et al.*: Acta Crystallogr. F **63** (2007) 678.