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ZupT Is a Zn(II) Uptake System in *Escherichia coli*

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***Escherichia coli zupT* (*ygiE*), encoding a ZIP family member, mediated zinc uptake. Growth of cells disrupted in both *zupT* and the *znuABC* operon was inhibited by EDTA at a much lower concentration than a single mutant or the wild type. Cells expressing ZupT from a plasmid exhibited increased uptake of $^{65}Zn^{2+}$.**

Zinc is an essential transition metal for all organisms and serves as a cofactor in members of all six major functional classes of enzymes (18). It also serves as a structural cofactor for many proteins. As a result, organisms have developed mechanisms for maintaining adequate concentrations of intracellular zinc while preventing metal ion overaccumulation. Cells of *Escherichia coli* encounter fluctuating extracellular zinc levels and maintain zinc homeostasis by transporting excess metal out of the cell and regulating zinc uptake across the cytoplasmic membrane. Prior to this report, three zinc transport systems had been characterized for *E. coli*. Efflux of zinc is accomplished by the P-type ATPase ZntA (2, 16) and the cation diffusion facilitator ZitB (7). Under conditions of deficiency, zinc is taken up by the high-affinity ABC transporter ZnuABC (14). In this report we show that ZupT is an additional zinc transporter responsible for zinc uptake. ZupT is the first characterized bacterial member of the ZIP family of proteins, previously only reported to be present in eukaryotes. The ZIP family derived its name from the first identified members (ZRT, IRT-like protein) (9). These transporters were initially identified as iron or zinc transporters but were subsequently shown to be able to also transport manganese and cadmium. However, the specificity and affinity to different metals are specific for each ZIP transporter (6, 9).

Either ZupT (YgiE) or ZnuABC is necessary for growth under zinc-limited conditions. ZnuABC had been shown to be responsible for high-affinity zinc uptake (14). However, addition of EDTA to mineral salts medium did not completely abolish growth of *E. coli* strain GR352 Δ*znuABC*::*cam*, indicating the presence of other transporters. Recently, a review by Gaither and Eide (6) suggested that the ZIP family is not restricted to eukaryotes but is also present in bacteria. In *E. coli* the *ygiE* gene encodes a putative ZIP protein (6). To assess the physiological role, *ygiE* was deleted as described previously (4). Deletion of *ygiE* in *E. coli* GR316 (Δ*ygiE*::*cam*) only slightly affected growth under the conditions tested (Fig. 1A). However, growth of the double mutant *E. coli* GR354 (Δ*znuABC* Δ*ygiE*::*cam*) with both *ygiE* and *znuABC* deleted was severely inhibited by the presence of EDTA. This inhibition was more pronounced in the double mutant than in the single mutants *E. coli* GR352 (Δ*znuABC*::*cam*) and GR316 (Δ*ygiE*::*cam*) (Fig. 1A). Addition of zinc but not of nickel, copper, or cadmium alleviated this inhibition (Fig. 1B), indicating that *ygiE* is responsible for zinc uptake. We therefore renamed *ygiE* as *zupT* to indicate its role as a zinc uptake transporter. ZnuABC appears to have a higher affinity for zinc than ZupT, since a strain with a deletion of *zupT* is less inhibited in growth by the addition of high concentrations of EDTA than a strain with a deletion of *znuABC*. *E. coli* GR352 (Δ*znuABC*) showed improvement of growth after addition of zinc but also a slight increase after nickel addition (Fig. 1B). This could be caused by slight impurities in the nickel salt used. However, zinc clearly is the growth-limiting factor in the *E. coli* GR352 (Δ*znuABC*) single mutant and the *E. coli* GR354 (Δ*znuABC* Δ*ygiE*::*cam*) double mutant.

Expression of ZupT makes cells zinc hypersensitive. To further characterize ZupT-mediated metal transport, ZupT was cloned into the inducible expression vector pASK-IBA3 (IBA, Göttingen, Germany), creating pZUPT. Expression of *zupT* was induced by the addition of 200 ng of anhydrotetracycline (AHT) (Sigma-Genosys)/ml, which made cells hypersensitive to zinc (Fig. 2A). This effect was most pronounced in strain *E. coli* GG48, in which the *zntA* and *zitB* genes, encoding zinc efflux pumps, had been disrupted. Expression of *zupT* in *E. coli* GG48 (*zntA*::*kan* Δ*zitB*::*cam*) even led to reduced viability in Luria-Bertani (LB) broth without added zinc due to residual zinc present in the medium (Fig. 2A). However, addition of EDTA alleviated the toxic effects of expression of *zupT* (Fig. 2B). This indicated that zinc hypersensitivity is caused by overaccumulation of cytosolic zinc and is not due to possible toxic effects of protein overproduction. Furthermore, a slight increase in copper sensitivity in wild-type cells expressing *zupT* was observed, indicating that ZupT transports copper in addition to zinc (Fig. 2C). In contrast, addition of cadmium to cells expressing *zupT* did not lead to a pronounced increase in sensitivity as observed with zinc (Fig. 2D). On the contrary, the addition of 5 μM Cd(II) led to an increase in viability over that for cells in LB broth without added metal, indicating that Cd(II) might inhibit uptake of zinc by ZupT. This is in agreement with previously reported metal specificities of ZIP proteins from *Arabidopsis thaliana* (8).

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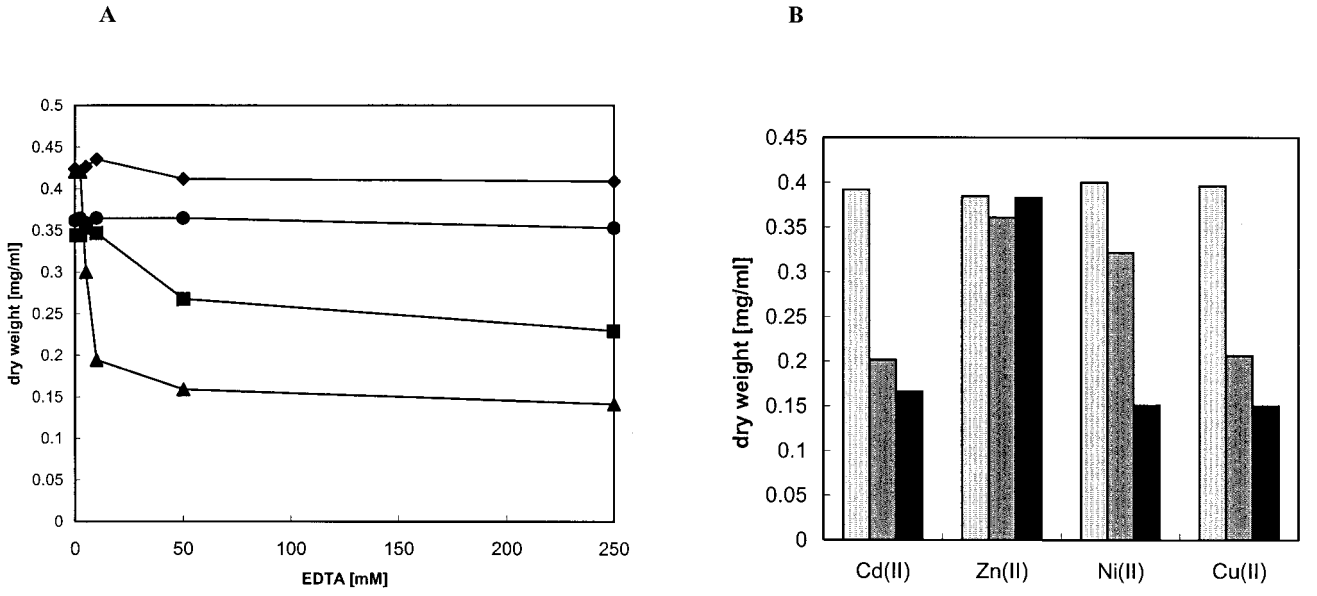

FIG. 1. Effect of zinc depletion on growth of *E. coli* W3110, GR352 (Δ*znuABC*::*cam*), and GR354 (Δ*zupT*::*cam* Δ*znuABC*). Growth yields with different EDTA concentrations are shown. (A) Overnight cultures grown in LB medium were diluted 1:400 into fresh mineral salts medium (12) with the indicated concentrations of EDTA. Cell growth was monitored by measuring the OD_{600} after 6 h of incubation at 37°C with shaking and converted to dry weight. Results are shown for *E. coli* W3110 (◆), *E. coli* GR316 (Δ*zupT*::*cam*) (●), *E. coli* GR352 (Δ*znuABC*::*cam*) (■), and *E. coli* GR354 (Δ*zupT*::*cam* Δ*znuABC*) (▲). Experiments were performed in triplicate, and the average was calculated. (B) Cells were treated as for panel A except that equimolar amounts of EDTA and metals were added (both at 250 μM). Results are shown for *E. coli* W3110 (light grey bars), *E. coli* GR352 (Δ*znuABC*::*cam*) (dark grey bars), and *E. coli* GR354 (Δ*zupT*::*cam* Δ*znuABC*) (black bars).

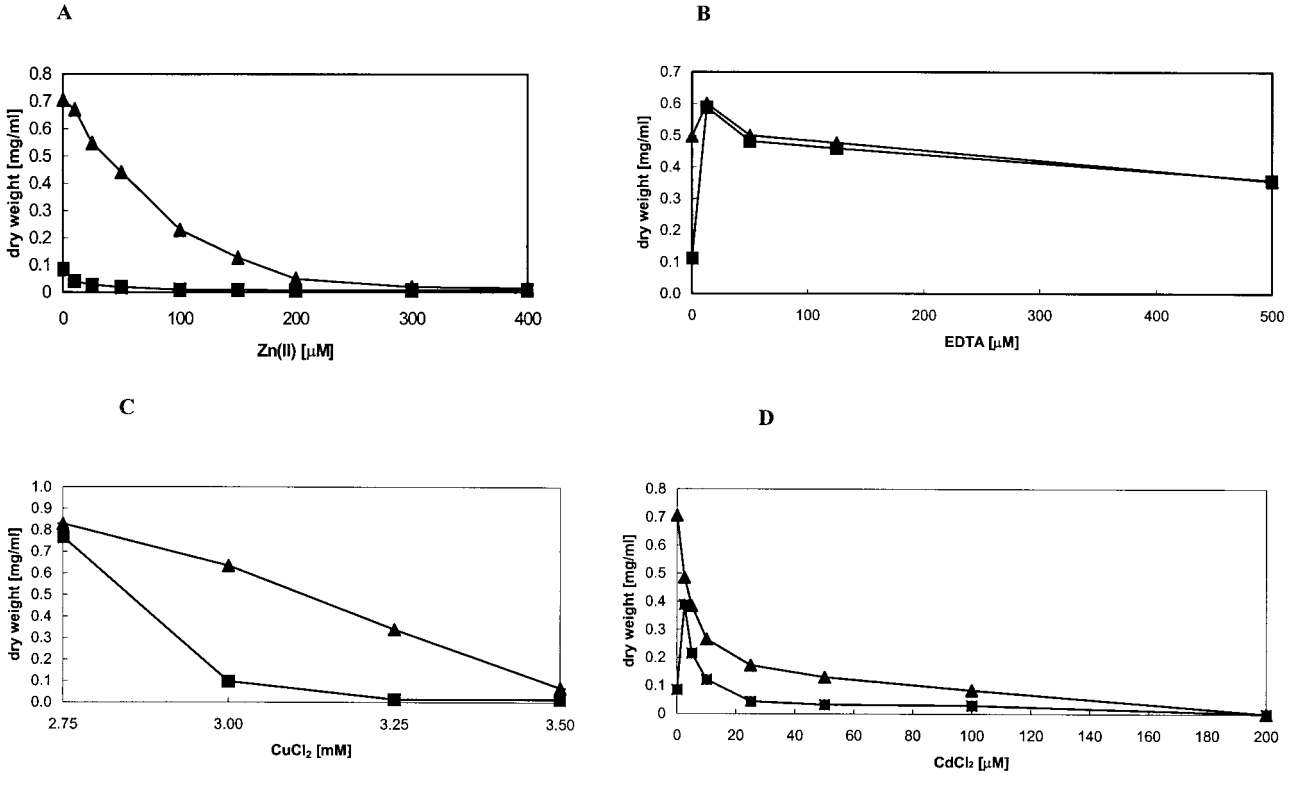

FIG. 2. Growth of *E. coli* strains expressing *zupT* in the presence of metals or EDTA. Overnight cultures grown in LB medium were diluted 1:400 into fresh LB medium with the indicated concentrations of zinc (A), EDTA (B), copper (C), or cadmium (D), and expression of *zupT* was induced with 200 ng of AHT/ml. Growth was monitored after 6 h by measuring the OD_{600} and converted to dry weight (mg/ml). (A, B, D) ■, *E. coli* GG48 (Δ*zntA*::*kan* Δ*zitB*::*cam*)(pZUPT); ▲, *E. coli* GG48 (Δ*zntA*::*kan* Δ*zitB*::*cam*) vector control. (C) ■, *E. coli* W3110(pZUPT); ▲, *E. coli* W3110 vector control. Results of representative experiments are shown.

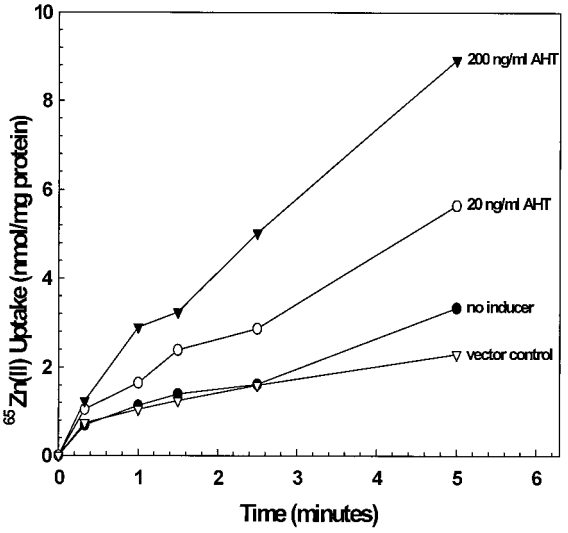

FIG. 3. Uptake of ^{65}Zn(II) by cells of *E. coli* GR362 (Δ*znuABC* Δ*zupT* Δ*zntB*::*cam* Δ*zitB zntA*::*kan*) expressing zupT. Cells were grown overnight in LB medium, diluted 50-fold into fresh prewarmed LB medium, and grown to an OD_{600} of 0.5, and expression of *zupT* was induced with 200 or 20 ng of AHT/ml for 10 min or not induced. The cells were washed with buffer A (10 mM Tris-HCl [pH 7.0], 2 g of glucose/liter, 10 mM Na_2HPO_4) and resuspended in the same buffer. $^{65}ZnSO_4$ was added to a final concentration of 10 μM. The cells were incubated at 37°C, and 0.1-ml aliquots were filtered through nitrocellulose membranes (pore size, 0.45 μm) at various times and immediately washed with 10 ml of buffer B (10 mM Tris-HCl [pH 7.0], 10 mM $MgCl_2$). The membranes were dried, and radioactivity was measured using a liquid scintillation counter. The protein concentration was determined using the method of Lowry et al. (11), and the amount of Zn(II) per milligram of protein was calculated. ●, *E. coli* GR362 (Δ*znuABC* Δ*zupT* Δ*zntB*::*cam* Δ*zitB zntA*::*kan*)(pZUPT) (no inducer); (○), E. coli GR362 (Δ*znuABC* Δ*zupT* Δ*zntB*::*cam* Δ*zitB zntA*::*kan*) (pZUPT) (20-ng/ml AHT); ▼, E. coli GR362 (Δ*znuABC* Δ*zupT* Δ*zntB*::*cam*Δ*zitB zntA*::*kan*)(pZUPT) (200 ng/ml); ▽, *E. coli* GR362 (Δ*znuABC* Δ*zupT* Δ*zntB*::*cam* Δ*zitB zntA*::*kan*)(pASK-IBA3).

ZupT mediates uptake of ^{65}Zn. To measure uptake of $^{65}Zn^{2+}$ by ZupT, all known zinc transport systems in *E. coli* were disrupted (4). The *zntA* and *zitB* genes both encode efflux systems (7, 16), whereas *znuABC* and *zupT* are responsible for zinc uptake (14; this report). In addition, the *zntB* gene, encoding a putative zinc efflux system, was deleted (data not shown). This strain, *E. coli* GR362 (*zntA*::*kan* Δ*zitB* Δ*zupT* Δ*znuABC* Δ*zntB*::*cam*), showed normal growth in LB medium and was transformed with the plasmid pZUPT. Expression of *zupT* was induced with different concentrations of AHT for 10 min. Cells with the largest amount of inducer added (200 ng/ml) showed the largest increase in zinc uptake compared to the vector control, *E. coli* GR362(pASK-IBA3) (Fig. 3). Addition of smaller amounts of inducer still led to a significantly higher level of accumulation of $^{65}Zn^{2+}$ than was found with control cells (Fig. 3).

Conclusions. In this report we show that ZupT is responsible for zinc uptake in *E. coli*. ZupT represents the first bacterial member of the ZIP family (5, 6, 9). Therefore, the transporters involved in zinc homeostasis in *E. coli* constitute a mixture of uniquely bacterial systems, such as ZnuABC and transporters that belong to ubiquitously distributed protein families, such as the CDF member ZitB (13, 15) and the ZIP protein ZupT. P1-type ATPases such as ZntA have also been identified but not yet characterized in plants (1), indicating that this family is also present in all three kingdoms. In addition to these well-characterized metal transporters, zinc might also be able to enter as a metal phosphate via the Pit phosphate uptake system (3), but this appears to be adventitious. Since zinc is such an important nutrient, it is not surprising to observe redundancy in transport systems. However, the number and families of putative zinc transporters are not conserved in different bacteria and might reflect different physiological needs. In addition, metal transporters sometimes seem to be acquired by horizontal gene transfer, since the sequences of transporters often do not follow the overall phylogenetic patterns of the particular microbes as determined by sequence comparison of small-subunit rRNAs (10, 17, 17a).

Finally, it appears likely that ZupT is a broad-range metal ion transporter in *E. coli*, perhaps mediating transport of other divalent cations including Cd(II) and Cu(II). It demonstrably transports Zn(II); Cd(II) antagonizes the effect of Zn(II) in a *zupT*-overexpressing strain; and cells expressing ZupT exhibit a slight increase in Cu(II) sensitivity.

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