EFFLUX SYSTEMS IN METALLOPHILES

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Contents

Summary

Metal efflux in metallophiles is accomplished by a multitude of different transporters. P-type ATPases are primary transporters because they are driven by ATP-hydrolysis and thus are highly efficient. Secondary or additional transporters belong to the resistance nodulation cell division (RND), the cation diffusion family (CDF) or the Major Facilitator Superfamily (MFS). Members of these families are probably proton driven. RND proteins are the core of a transport complex that encompasses both the cytoplasmic and outer membrane. CDF and MFS proteins transport metals from the cytoplasm across the cytoplasmic membrane into the periplasmic space. Virtually all microorganisms possess metal efflux systems. Metallophiles however, excel in both, the abundance of members of different families of transporters and the multitude of redundant members of the same family. Mobile DNA that is frequently rearranged to create novel combinations of metal resistance determinants might be an adaptation to survive in environments with extremely high metal concentrations.

1. Introduction

Geochemical cycles operating near Earth's surface have been altered fundamentally by microbial metabolisms ever since the origin of life. Life evolves in a context defined by physical and chemical surroundings that are constantly changing. Since the environment where life developed was probably rich in divalent metals, mechanisms for metal homeostasis are a requirement for life. The goal of this chapter is to describe bacterial divalent metal efflux transporters and their role in metal homeostasis integrating recent findings. Since the transporters responsible for metal efflux are similar in metal

tolerant/metallophile bacteria such as *Ralstonia metallidurans* CH34 and "normal" bacteria such as *Escherichia coli* or *Bacillus subtilis*, the transporters will be discussed as related protein families.

Microorganisms need to take up metal cations such as Co(II), Cu(I) or Cu(II), Fe(II) or Fe(III), Mn(II), Ni(II), and Zn(II) because they are required as micronutrients for vital cell functions. However, these metal cations are also toxic in high concentrations, making homeostatic resistance mechanisms necessary. In the last five years, it has become obvious that the differentiating between plasmid-borne resistance mechanisms and intrinsic chromosomal determinants responsible for metal homeostasis and trafficking is not as sharp as it once seemed. The same cell frequently contains both a nutrient uptake and a homeostasis efflux transport system for the same cation. This is well illustrated in the bacterial model organism *E. coli*. There are at least two transport systems responsible for zinc uptake and two to ensure efflux of excess zinc (Figure 1).

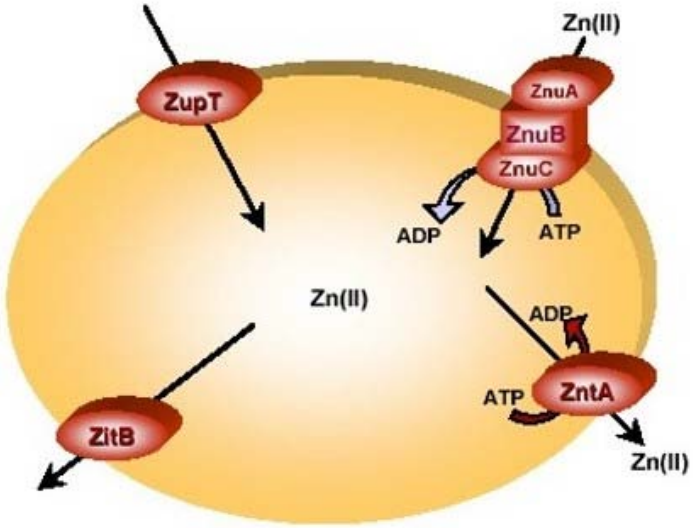

Figure 1. Zinc transport systems in *E. coli*
In this model the characterized zinc transport system are shown. Under conditions of zinc deficiency zinc is taken up by ZupT (the *ygiE* gene product) and ZnuABC. ZupT belongs to the ZIP family of metal transporters and ZnuABC is an ABC transport system. Uptake systems are not discussed in this chapter. Zinc-translocating efflux pumps are the P-type ATPase ZntA and the CDF protein ZitB. Interestingly, *E. coli* seems to have at least two transporters responsible for uptake and for efflux. ZnuABC and ZntA are both ATPases and are probably more powerful high affinity transporters than ZitB or ZupT who might be responsible for zinc homeostasis under physiological conditions.

Metal transporters are members of one of three major transporter groups: P-type ATPases, ABC multicomponent ATPases, and membrane potential-driven non-ATPase transporters. Looking just at genes for divalent cation transporters, the rapidly increasing number of completed microbial genomes often contain close homologues on the chromosomes to previously identified plasmid-borne resistance determinants. For

example, the genes for divalent cation efflux transporters such as homologues of the *Escherichia coli* chromosomal genes *zntA* or *copA* sometimes seem to be acquired by horizontal gene transfer. This observation is underscored by the observation that sequences cluster by cation specificity and function. They often do not follow the overall phylogenetic patterns of the particular microbes, as determined by sequence comparison of small subunit ribosomal RNAs. Rather, cation transporters are an example of where genes appear to have been exchanged in the course of evolution and selection by lateral gene transfer. A goal is to be able to predict accurately the cation specificity and direction (uptake or efflux) for members of cation transporters, based on sequence without resort to direct experiment. This goal has not been achieved. However, certain motifs and domains are conserved in divalent cation transporters, regardless of microbial ancestry. Recently it was shown that intracellular levels of zinc and copper are extremely low. Therefore, homeostatic mechanisms for divalent cations are widely found and required for life in rapidly fluctuating environments such as soils and waters, making it likely that transporters for divalent cations arose early in evolution.

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Biographical Sketches

Christopher Rensing studied biology and chemistry at the FU Berlin. His diploma thesis described the functional expression of a bacterial mercury reductase in yeast and was completed in 1992 under the supervision of Dr. Bärbel Friedrich. After following his PhD advisor Dr. Dietrich Nies from Berlin to the Martin-Luther Universität Halle-Wittenberg, the focus of his work shifted from eukaryotic to bacterial systems. In 1996, he was awarded the doctoral degree for his work on the characterization of the cobalt-zinc-cadmium (*czc*) determinant from *Ralstonia metallidurans* CH34. Dr. Rensing then spent three years as a postdoc in the laboratory of Dr. Barry Rosen at Wayne State University in Detroit. His postdoctoral research included the biochemical characterization of two soft metal transporting P-type ATPases in *E. coli*. Currently he is an assistant professor in the Department of Soil, Water, and Environmental Sciences at the University of Arizona.

Gregor Grass studied biology at the Martin Luther Universität Halle-Wittenberg, Germany. In 1996, he completed his MSc in which he elucidated the transcriptional regulation of the *czc*-determinant from *Ralstonia metallidurans* CH34 under the supervision of Dr. Dietrich Nies. His PhD thesis under continued supervision of Dr. Dietrich Nies centered on the molecular genetic and biochemical characterization of the cobalt/nickel (*cnr*) resistance determinant from *Ralstonia metallidurans* CH34, for which he was awarded the doctoral degree in 2000. Since then, he has worked as a postdoc in the laboratory of Dr. Christopher Rensing in the Department of Soil, Water, and Environmental Sciences at the University of Arizona.