New Method Tracks Metal-ion Movement in Periplasmic Proteins

Copper is an essential nutrient for most organisms. However, it is toxic at high concentrations and, in fact, is used by macrophages to kill invading microbes. To counter the lethal effects of both environmental and host-defense onslaught, bacteria have devolved intricate efflux systems that impart full copper resistance.[1] The periplasmic CusCBA pump in *Escherichia coli* is one example of this type of efflux system (Figure 1). The Cus system is structurally similar to the bacterial RND-type (resistance nodulation division) drug efflux complexes with some striking differences in (1) the involvement of its periplasmic adapter protein CusB, which binds Cu^+ and Ag^+, and (2) the co-expression of the Cu^+/Ag^+ metallochaperone CusF.[2]

The mechanism of shuttling toxic metal ions out of the periplasm by the Cus system has been difficult to determine by biochemical and molecular techniques alone because it is composed of four distinct proteins – three of them have been shown to bind and release metal ions. Two scenarios have been proposed: (1) a relay model, in which metal ions travel from CusF to CusB to CusA, and (2) a switch model, in which the metal-loaded CusB adaptor protein serves to activate CusA to accept Cu^+ or Ag^+ directly from CusF.[3]

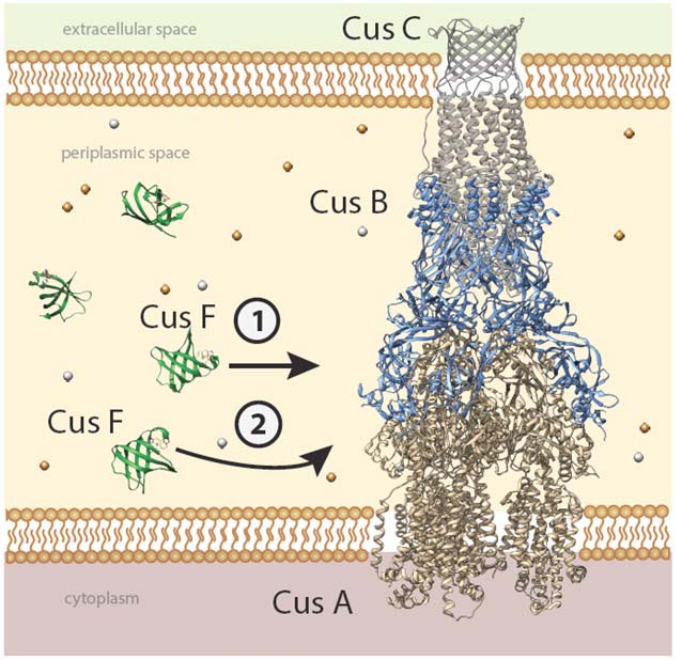

Figure 1. Cartoon overview of the CusCBFA efflux pump in the periplasm.

To better track the individual functions of the Cus proteins during Cu^+ and Ag^+ efflux, a team led by researchers from Oregon Health & Science University has used a new technique that they had previously pioneered.[4] They replaced the protein active site methionine (Met) with selenomethionine (SeMet) residues and subsequently studied dilute metalloprotein interactions by x-ray absorption spectroscopy (XAS). By observing changes of the selenium–metal interactions in the extended x-ray absorption fine structure (EXAFS) of the Se-*K*-edge, specific holo and apo metal sites within a mixture of proteins can be clearly distinguished (Figure 2a).

All three of the Cus proteins under investigation contain at least two active site Met residues, making it possible to combine all three Cus proteins together *in vitro,* as long as only one of them (CusF or CusB) is labeled with SeMet. In this manner the scientists were able to track the metal gain or loss by each SeMet-labeled Cus component. The team also utilized the Cus system's ability to transport either Ag or Cu ions.

The researchers first determined that fully metal-loaded CusB and CusF cannot exchange metal ions with each other unless one of them is in the apo form. This finding allowed the

team to include fully copper-loaded CusF, fully silver-loaded CusB and apo CusA in the same reaction and use the unique SeMet label to track metal transfer in a series of tri-metal (Cu, Se and Ag) XAS experiments at SSRL Beam Lines 7-3 and 9-3 at relatively low concentrations (~150μM).

By analyzing the Se-*K*-edge spectra of the mixture of SeMet-labeled Ag-loaded CusB, unlabeled Cu-loaded CusF and apo CusA, the researchers found that the periplasmic adapter protein CusB was not able to transfer metal ions directly to CusA, ruling out a relay role for CusB.

When the scientists analyzed the alternate tri-protein mixture in which Cu-loaded CusF was SeMet-labeled, they observed that CusF released all of its bound Cu^+, but only when CusB was present and fully loaded with Ag^+ ions (Figure 2b). Additionally, they found that when CusF released its Cu^+, back transfer of Ag^+ from CusB was observed, consistent with earlier observations that metal exchange between apo and holo CusF and CusB is fully reversible. This implies a "switch" role for CusB and suggests that access to the CusA pump is controlled by the metal status of CusF and hence that of the periplasm as a whole.

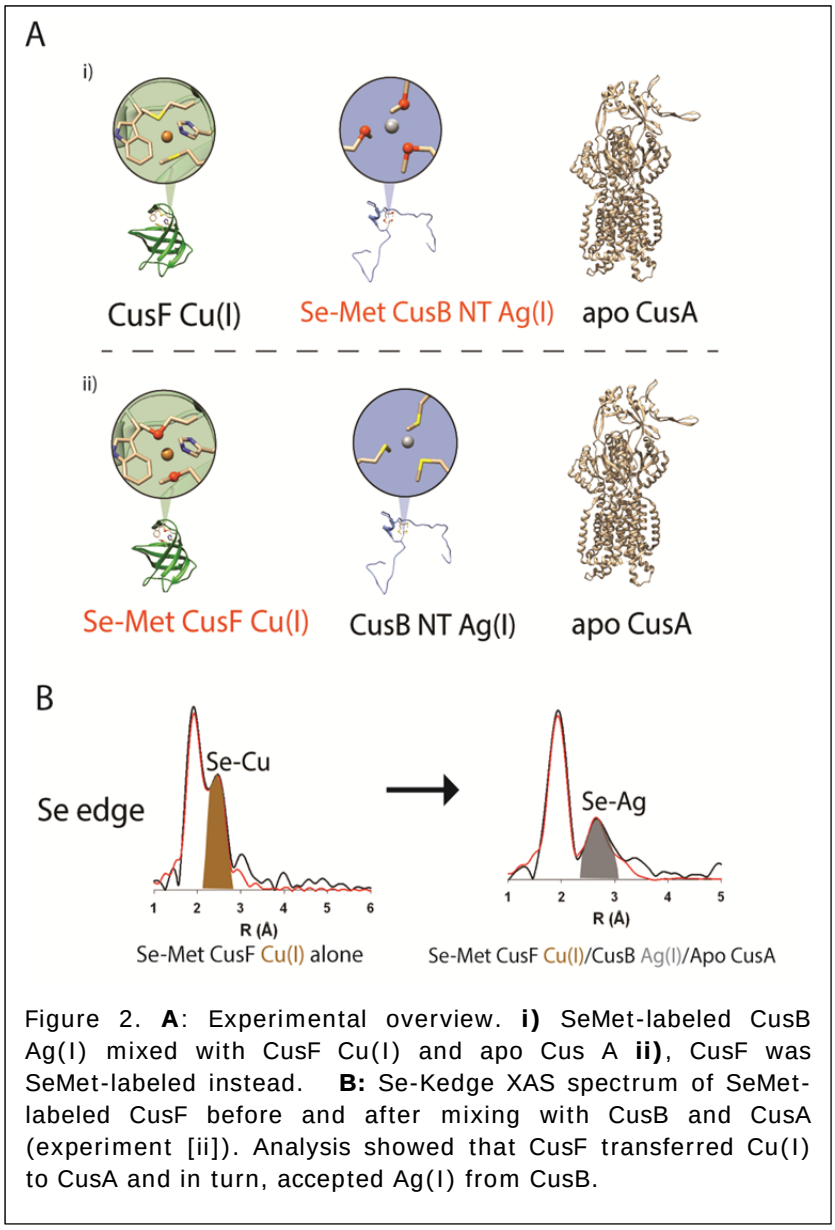

Figure 2. **A**: Experimental overview. **i)** SeMet-labeled CusB Ag(I) mixed with CusF Cu(I) and apo Cus A **ii)**, CusF was SeMet-labeled instead. **B:** Se-Kedge XAS spectrum of SeMet-labeled CusF before and after mixing with CusB and CusA (experiment [ii]). Analysis showed that CusF transferred Cu(I) to CusA and in turn, accepted Ag(I) from CusB.

To interrogate the structure of the final destination of Cu it was necessary to analyze the EXAFS at the Cu-*K*-edge of the mixture. The team observed that all of the Cu from CusF had moved to occupy a capture complex in CusA with an unusual $(Met)_2O$ ligand set.

These results are an important first step in understanding the structural basis of the mechanism through which pathogenic bacteria resist otherwise toxic levels of metal ions at the host–pathogen interface. They are also a strong proof-of-concept for this type of multi-edge, SeMet-labeling technique. Future studies will focus on unravelling further details of the mechanism with the goal of evaluating these unique bacterial efflux pumps as possible targets for antimicrobial drugs.

Primary Citation

K. N. Chacón, T. D. Mealman, M. M. McEvoy and N. J. Blackburn, "Tracking Metal Ions through a Cu/Ag Efflux Pump Assigns the Functional Roles of the Periplasmic Proteins", *Proc. Natl. Acad. Sci. USA* **111**, 15373 (2014), DOI: 10.1073/pnas.1411475111

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SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Institute of General Medical Sciences.