ABSTRACT

The goal of this proposal is to define the mechanisms of Cu+ homeostasis in the pathogen Pseudomonas aeruginosa. This organism is an important and frequent cause of hospital acquired infection, especially in immune compromised patients. Cu+ is a central element in host-pathogen interactions. It is a micronutrient required as a redox co-factor in the catalytic centers of enzymes. However, free Cu+ is highly reactive and deleterious to cells. It is not known how cellular components interact and participate in ion distribution equilibria to achieve tolerance to high Cu+ concentrations and Cu+ targeting to essential cuproenzymes. We hypothesize that cells have two Cu+ sensing/distribution networks. One is responsible for targeting Cu+ to cuproproteins and responds to changes in cuproenzymes functionality when challenged by environmental stressors. The other network maintains a cellular Cu+ quota and responds to cytoplasmic Cu+ levels. We aim to define and model the Cu+ distribution networks and their dynamic response to stress. We propose to characterize the specificity and routes of Cu+ entrance, distribution in the cytoplasm, transport t the periplasm, and final targeting to efflux systems or cuproenzymes. To this end, compartmental fluxes will be characterized in combination with in vivo biochemical equilibria amongst Cu+-sensing and distributing molecules. Systems of differential equations will integrate obtained parameters into mechanistically driven mathematical models. These will be experimentally validated. Departing from reductionist approaches, the project will generate a shift in the analysis of heavy metal homeostasis by considering the full range of involved elements, the biochemical equilibria in which they participate, and the integrated system response to environmental challenges. The approach will be applicable to other bacterial systems and micronutrient biometals.