

Soil Bacteria Transport Systems: Ligand and Regulatory Specificity

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Project Goals: The Argonne “Environment Sensing and Response” Scientific Focus Area (SFA) program seeks to identify the molecular basis of cellular transport and sensory pathways that mediate the response of terrestrial ecosystems to environmental nutrients. The mechanistic links between and within ecosystems comprised of plants, fungi, and soil bacteria involved in the production of biomass for fuel are currently very poorly defined. The effects of nutrient availability, closely linked to climate, on those mechanistic links, are also inadequately understood. This program will address this knowledge gap by mapping transport and sensor proteins to specific environmental compounds to define their function and biological roles and establish a series of defined connections between the environment and the cell. The knowledge will facilitate the development of system-level models predictive of cellular response to changes in environmental conditions.

A typical bacterial ABC transporter contains three protein components: a solute binding protein (SBP), a transmembrane domain (TMD) dimer spanning the inner membrane, and an ATPase dimer in the cytoplasm (Fig. 1). In bacteria, roughly 2-5% of the genome codes for components of ABC type transporters (1). Certain types of soil bacteria contain an even higher percentage of ABC transporters, such as *Pseudomonas fluorescens*, a species that contains well over 350 transport protein components [as identified by Transport DB (1)]. Interestingly though, approximately 30% of the ABC transporter proteins in *P. fluorescens* PF-5 are ‘orphans’ not genomically co-located with other transporter component genes and thus difficult to assign to a specific complex. The abundance of ABC transporter component genes and the presence of orphan genes suggest the potential for crosstalk amongst members. While studies have indicated that TMDs can interact with multiple SBPs with varying affinity and specificity (2), there is limited information regarding the specificity of TMD and ATPase protein-protein interactions. The promiscuity of TMD-ATPase interactions may also affect promiscuity of SBP interactions with the transporter complex and refine ligand specificity of the overall transporter. To address this knowledge gap, the SBPs and the membrane-localized transmembrane components of complexes involved in methionine importation from a series of *Pseudomonas fluorescens* strains were investigated *in vitro*. Of significance to these experiments is that ligand binding of methionine to the ATPase domain in an *Escherichia coli* homolog of these systems (MetNI) causes trans-inhibition, where transport is suppressed in a concentration-dependent manner by the substrate.

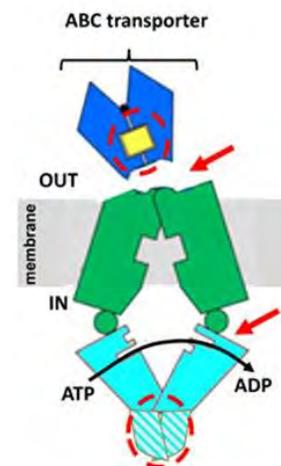


Figure 1. Model of ABC transporters involved in nutrient importation. Red, dashed circles indicate ligand binding sites and red arrows are interfaces where orphaned components may contribute via cross-talk.

Genomic analyses of four *Pseudomonas fluorescens* strains (PF-5, Pf0-1, SBW25, and WH6) have identified a set of ten ATP-binding cassette (ABC)-type amino acid transporters with high sequence similarity to the structurally characterized MetNI from *E. coli*. Recombinant, dual-vector expression as well as polycistronic strategies yield intact complexes of the TMD and ATPase components of the *P. fluorescens* gene targets in *E. coli*. This polycistronic approach allows for limiting tag interference in the complex as well as having more controlled TMD-ABC stoichiometry. Expression experiments systematically varying the combination of ATPase genes with one TMD gene revealed promiscuity of

certain ATPases, where stable “hybrid” complexes could be formed with both a TMD from the same strain as the ATPase or with a TMD from a different *P. fluorescens* strain. This type of functional “crosstalk” between ATPases and TMDs could play a role in rapid nutrient exchange between *Pseudomonas* soil bacteria and other rhizosphere inhabitants. The ability of different ATPases to recognize the same binding site of a TMD also provides an opportunity to study the molecular basis of recognition between the domains of the transporter core.

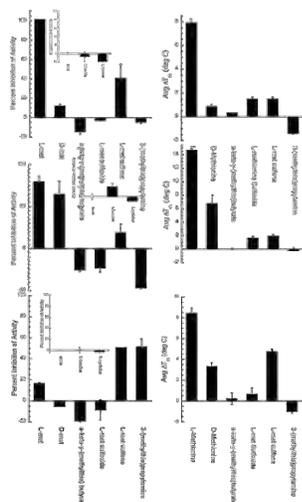


Figure 2. Similarities of ligand binding to SBPs (right panels) and ATPase regulatory domains (left panel) in ABC transporters involved in methionine uptake in soil bacteria. A, *E. coli* MetNI; *P. fluorescens* native ATPase; C, *P. fluorescens* orphaned ATPase.

Few ABC transporters with trans-inhibition regulatory features have been characterized so it is unknown if the regulatory small molecule always matches the transported small molecule. Utilizing ATPase activity inhibition assays, it is possible to identify ligands that have an inhibitory effect on transporter complex function. We determined probable transporter substrates by testing the specificity of associated MetQ-family SBPs using a fluorescence-based thermal shift assay. The ATPase inhibitors were compared with the SBPs ligand-binding profiles to determine if there were patterns between small molecules transported from the environment and the internal regulators of transport activity. Crosstalk at both the external and internal membrane interfaces may have a role in expanding transporter capacity while retaining specificity. Association of non-native ATPases with a TMD could also enable activity regulation by alternative regulatory substrates. Comparison of profiles from the activity inhibition assay and the thermal shift assay for a set of methionine-derivative ligands for native and non-native transporter complexes support these hypotheses (Figure 2). These results demonstrate how multiple complete transporters and orphan components may function together to afford functional advantages to bacteria in complex and highly competitive environments such as the plant rhizosphere. Approaches used for methionine import have been expanded for use in the understanding of how carbon is shuttled between community players within the rhizosphere.

References

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