



# PERIPLASMIC BINDING PROTEIN DEPENDENT ABC TRANSPORTERS ARE BI-DIRECTIONAL

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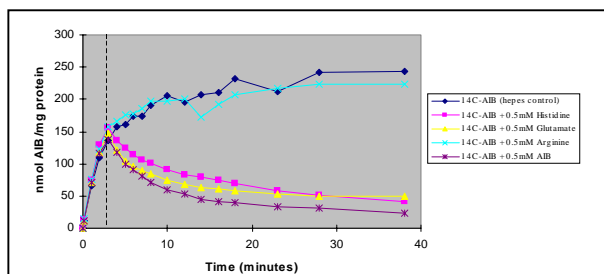
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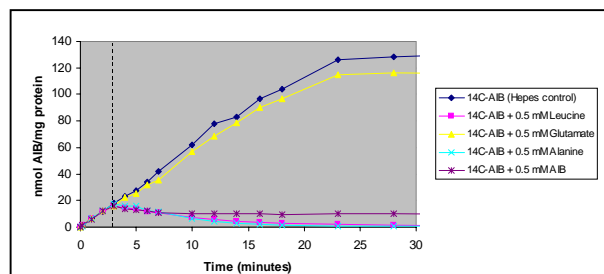
## Introduction

Members of the ATP-binding cassette (ABC) transporter superfamily are ubiquitous in both prokaryotes and eukaryotes. They are responsible for translocation of a wide range of solutes both into and out of cells. The periplasmic binding protein dependent ABC transporters are a subfamily of transporters, found only in prokaryotes, which are responsible for the uptake of solutes. The current models of ABC transporter function state that they can only transport solutes in one direction, i.e. into or out of cells. However, the findings of this study challenge this paradigm.

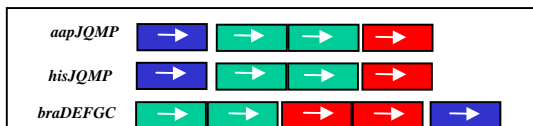
Three amino acid transporters belonging to the ABC family have been investigated with regard to their ability to transport amino acids into and out of bacterial cells (Figure 1). The general amino acid permease (Aap) of *R. leguminosarum* has been previously reported to exhibit a bi-directional phenotype (1). The His transporter of *S. typhimurium* is one of the most extensively studied ABC transporters (2). The third transporter used in this study is the previously undescribed Bra transporter of *R. leguminosarum*. The genes of this operon possess greatest homology to the Liv transporters of *E. coli* & *S. typhimurium* and the branched chain amino acid (Bra) transporter of *Pseudomonas aeruginosa*.



**Figure 2. Transport of <sup>14</sup>C-AIB by *R. leguminosarum* RU1099 pRU191.** Cells from an exponentially growing culture were harvested, washed and starved of carbon & nitrogen sources for 1 hour. Transport assays were initiated by adding α-aminoisobutyric acid (AIB) containing 0.5 μCi <sup>14</sup>C to a final concentration of 25 μM. After 2 min. 55 sec. competing solutes were added to a final concentration of 0.5 mM. Samples were removed at 1 min. intervals to determine the level of <sup>14</sup>C. RU1099 is a derivative of *R. leguminosarum* containing a deletion of *aapJQMP*; pRU191 is a multi-copy plasmid carrying *aapJQMP*.



**Figure 3. Transport of <sup>14</sup>C-AIB by *R. leguminosarum* RU1356.** Transport assays were performed as described in Figure 1. Strain RU1356 is *R. leguminosarum* 8401 pRL1JI Δ *aap*::Ω spec<sup>c</sup>.



**Figure 1. Operon structure of ABC transporters included in this study.** *R. leguminosarum aap* (X82596), *S. typhimurium his* (V01373) & *R. leguminosarum bra* (unpublished). Periplasmic binding proteins are represented by the blue box, integral membrane proteins by the green box and ATPases by the red box. The arrows indicate the direction of transcription.

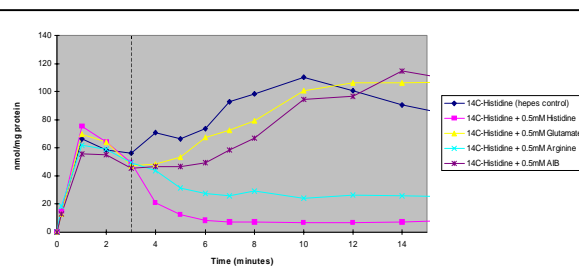
## Exchange of amino acids by *R. leguminosarum* Aap and Bra

A non-metabolisable solute of the Aap, α-amino isobutyric acid (AIB), was used to determine whether solutes taken up by *R. leguminosarum* could be exchanged for other solutes added in excess concentrations. The addition of AIB, glutamate or histidine caused the efflux of <sup>14</sup>C-AIB from pre-loaded cells (Figure 2). The rate of efflux is correlated to the copy number of *aap* (data not shown).

AIB is also a solute of the Bra transporter of *R. leguminosarum*. Exchange of AIB by Bra was investigated using an *aap*<sup>-</sup> strain (RU1356). Efflux of <sup>14</sup>C-AIB was observed following the addition of AIB, leucine or alanine, but not following the addition of glutamate (Figure 3). Therefore, when the Aap is the dominant transporter, glutamate can induce efflux of <sup>14</sup>C-AIB but it cannot when only Bra is present. Thus, the specificity of the transporter controls the specificity of exchange. No significant uptake of AIB is observed when both *aap* and *bra* were mutated in *R. leguminosarum* (data not shown).

## Exchange of amino acids by *S. typhimurium* His

Transport of histidine and arginine in *S. typhimurium* is enhanced when the copy number of the *his* operon is increased but uptake rates of other amino acids tested remain at wild type levels (data not shown). Correspondingly, only histidine and arginine induce efflux of <sup>14</sup>C-histidine from pre-loaded cells (Figure 4). As with the Aap of *R. leguminosarum*, the rate of efflux is dependent on the copy number of the transporter (data not shown).



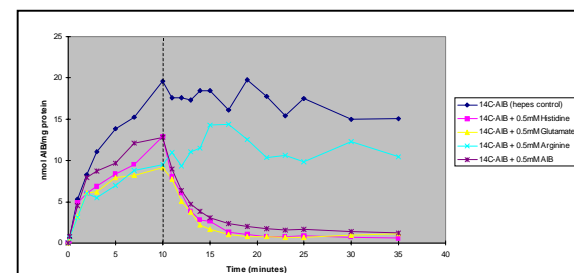
**Figure 4. Transport of <sup>14</sup>C-Histidine by *S. typhimurium* (NCIMB 10249) pRU607.** Transport assays were performed as described in Figure 1. Plasmid pRU607 is a multi-copy plasmid carrying *hisJQMP*.

## Heterologous expression of *R. leguminosarum* Aap in *S. typhimurium* supports the bi-directional hypothesis

Amino acid exchange is observed in the presence of three different ABC transporters. The rate of amino acid exchange is dependent on the copy number of the transporter. The specificity of exchange matches the specificity of efflux. Two hypotheses may explain this phenotype.

1. The ABC transporters are responsible for both uptake and efflux of solutes.
2. A specific export system regulated by the ABC transporters is responsible for efflux.

To determine whether the Aap of *R. leguminosarum* retains a bi-directional phenotype in a heterologous host, *aapJQMP* was cloned downstream of a *lac* promoter to facilitate expression in *S. typhimurium*. *S. typhimurium* is unable to transport AIB and does not possess a homologue of Aap (as determined by Southern blot; not shown). As in *R. leguminosarum* RU1099 pRU191 (Figure 2), glutamate, AIB and histidine induce efflux of <sup>14</sup>C-AIB from *S. typhimurium* pRU310, but arginine does not (Figure 5). It is extremely unlikely that Aap is regulating another transporter in a heterologous species, especially as the specificity of exchange is not altered. However, these data support the hypothesis that the Aap is responsible for the exchange of solutes.



**Figure 5. Transport of <sup>14</sup>C-AIB by *S. typhimurium* 10249 pRU310.** Transport assays were performed as described in Figure 1 with excess amino acids added at 9 min. 55 sec. Plasmid pRU310 is a multi-copy plasmid carrying *aapJQMP* under control of *plac*.

## Summary

Three amino acid transporters belonging to the periplasmic binding protein dependent ABC transporter subfamily apparently transport solutes in a bi-directional manner.

Evidence for this include:

- The rate of efflux is dependent on the copy number of the ABC transport operon.
- The specificity of solutes able to induce efflux of labeled solute correspond to the uptake specificity of the transporter.
- The bi-directional phenotype and exchange specificity is retained when the Aap is expressed in a heterologous host.

The current model of uni-directional transport by ABC transporters is therefore challenged. The significance of these findings to other ABC transporters, especially those of clinical importance (e.g. p-glycoprotein), awaits further investigation.

## References

1. Walshaw, D.L. & Poole, P.S. (1996) *Molecular Microbiology* **21** 1239-1252
2. Higgins, C.F., et al. (1982) *Nature* **298** 723-727