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Proton-dependent glutamine uptake by aphid bacteriocyte amino acid transporter ApGLNT1

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ABSTRACT

Aphids house large populations of the gammaproteobacterial symbiont *Buchnera aphidicola* in specialized bacteriocyte cells. The combined biosynthetic capability of the holobiont (*Acyrthosiphon pisum* and *Buchnera*) is sufficient for biosynthesis of all twenty protein coding amino acids, including amino acids that animals alone cannot synthesize; and that are present at low concentrations in *A. pisum*'s plant phloem sap diet. Collaborative holobiont amino acid biosynthesis depends on glutamine import into bacteriocytes, which serves as a nitrogen-rich amino donor for biosynthesis of other amino acids. Recently, we characterized *A. pisum* glutamine transporter 1 (ApGLNT1), a member of the amino acid/auxin permease family, as the dominant bacteriocyte plasma membrane glutamine transporter. Here we show ApGLNT1 to be structurally and functionally related to mammalian proton-dependent amino acid transporters (PATs 1–4). Using functional expression in *Xenopus laevis* oocytes, combined with two-electrode voltage clamp electrophysiology we demonstrate that ApGLNT1 is electrogenic and that glutamine induces large inward currents. ApGLNT1 glutamine induced currents are dependent on external glutamine concentration, proton (H^+) gradient across the membrane, and membrane potential. Based on these transport properties, ApGLNT1-mediated glutamine uptake into *A. pisum* bacteriocytes can be regulated by changes in either proton gradients across the plasma membrane or membrane potential.

1. Introduction

Animals live in a microbial world, and many animals form long-lasting beneficial associations with microbial partners that facilitate biodiversity [1,2]. An estimated 10% of all insect species harbor obligate bacterial endosymbionts in specialized cells and tissues [3]. Bacterial symbionts provide the host with novel metabolic pathways that allow host insects to exploit otherwise inaccessible niches. For example, blood-feeding insects (such as tsetse flies, bedbugs and body lice) have obligate bacterial symbionts that provide essential vitamins that are absent from their blood meal diet [4–7]. Similarly, plant phloem-feeding insects (such as aphids, psyllids, and mealybugs) have obligate bacterial symbionts that provide essential amino acids and vitamins that are absent or at low concentrations in their plant phloem sap diet [4,8–10].

Currently, the best-characterized insect nutritional symbiosis is that of the pea aphid, *Acyrthosiphon pisum* and its gammaproteobacterium *Buchnera aphidicola* [11,12]. *Buchnera* symbionts are housed in specialized aphid bacteriocyte cells located in the aphid hemoceol. Bacteriocyte cells (collectively forming the bacteriome) contain large *Buchnera* populations, with each symbiont partitioned from the bacteriocyte cytoplasm by an aphid derived symbiosomal membrane [13] (Fig. 1a). Extensive coevolution between *A. pisum* and *Buchnera* has had a dramatic effect on biological organization and complexity — such that the host and symbiont are metabolically integrated and function as an inseparable unit [11,14,15].

Large-scale sequencing and metabolic reconstruction of the *A. pisum*/*Buchnera* holobiont is beginning to shed light on the metabolic contribution of each partner and the nature of their molecular interdependency [8,11,14,16,17]. Adoption of an intracellular lifestyle rendered many *Buchnera* genes either functionally redundant, or not essential for maintenance of the symbiosis. Accumulation of deleterious mutations in these redundant genes, with eventual elimination from the *Buchnera* genome resulted in an extremely small, compact, gene-poor genome compared to their closest free-living relatives [8]. *Buchnera* retain genes for biosynthesis of ten amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), amino acids that aphids cannot synthesize and that are present at low concentrations

Abbreviations: AAAP, amino acid/auxin permease; APC, amino acid–polyamine–organocation; ApGLNT1, *Acyrthosiphon pisum* glutamine transporter 1; PAT, proton-dependent amino acid transporter; SLC, solute carrier; TEVC, two-electrode voltage clamp; V-ATPase, vacuolar-type H^+-ATPase.

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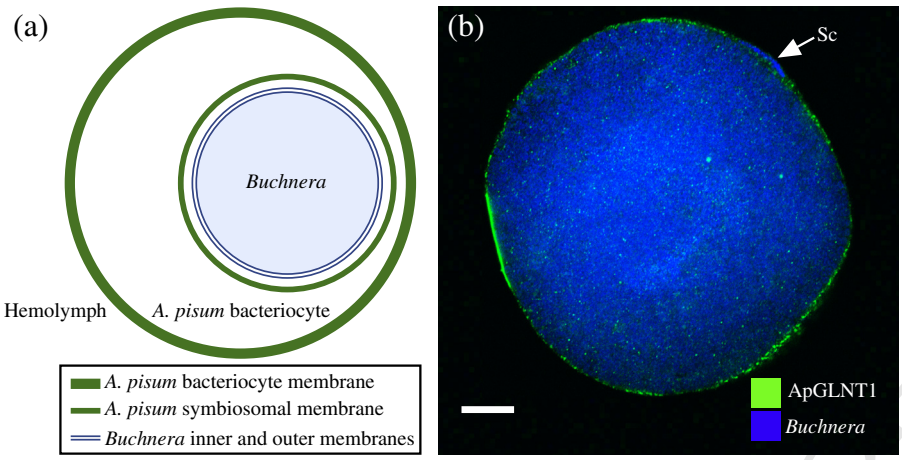

Fig. 1. Aphid bacteriocyte cellular organization and localization of ApGLNT1. (a) *A. pisum* bacteriocyte cell with an intracellular *Buchnera* symbiont (for simplicity a single symbiont is shown, not drawn to scale). (b) A single *A. pisum* bacteriocyte cell showing spatial localization of ApGLNT1 (green); DAPI-stained DNA of host cell and *Buchnera* (blue). Sc, sheath cell. Scale bar, 20 μm.

in *A. pisum*'s plant phloem sap diet [8]. Complementary *A. pisum* amino biosynthesis genes, which complete missing or fragmented *Buchnera* biosynthesis pathways are highly expressed in bacteriocyte cells [12,14,18]. Collectively, the combined biosynthetic capability of *A. pisum* and *Buchnera* is sufficient for biosynthesis of all twenty protein-coding amino acids [11,14,15]. Collaborative host and symbiont amino acid biosynthesis within bacteriocyte cells is facilitated by *A. pisum* amino acid transporters. *A. pisum* bacteriocyte plasma membrane amino acid transporter/s import precursor amino acids into bacteriocyte cells. Additional transporter/s at the symbiosomal membrane, a host derived membrane that partitions *Buchnera* from nutrient pools in the bacteriocyte cell, connect complementary host and symbiont amino acid biosynthesis pathways (Fig. 1a and electronic supplementary material, Fig. S1).

The *A. pisum* genome contains 40 putative amino acid transporters, 22 belonging to the amino acid/auxin permease (AAAP) family and 18 belonging to amino acid–polyamine–organocation (APC) family [19]. Of these, 4 AAAP family transporters (ACYPI000536, ACYPI000550, ACYPI001018 and ACYPI008971) and a single APC family transporter (ACYPI008904) are highly expressed and/or enriched in *A. pisum* bacteriocyte cells [19,20]. Recently, we functionally characterized transporter ACYPI001018 as a glutamine transporter, which we named *A. pisum* glutamine transporter 1 (ApGLNT1) [20]. ApGLNT1 has very narrow substrate selectivity, transporting only glutamine and localizes to the bacteriocyte plasma membrane [20]. Cellular localization and transport specificity is consistent with ApGLNT1 importing glutamine, the dominant hemolymph amino acid, into the bacteriocyte cell [21]. Importantly, glutamine transport is inhibited by arginine (a *Buchnera* synthesized amino acid) providing a potential mechanism by which bacteriocyte amino acid biosynthesis can be regulated by a *Buchnera* synthesized amino acid [20].

Here, based on the observation that ApGLNT1 is phylogenetically related to mammalian AAAP family proton-dependent amino acid transporters (PATs 1–4) [19,22] we investigate the electrogenic transport properties of ApGLNT1. A comprehensive review of AAAP family transporters [also known as solute carrier 36 family transporters (SLC36)] can be found in Thwaites and Anderson [23,24]. Using functional expression of ApGLNT1 in *Xenopus laevis* oocytes, coupled with two-electrode voltage clamp (TEVC) electrophysiology we confirm that ApGLNT1 is specific for glutamine, and that glutamine induces large inward currents. Furthermore, we demonstrate that glutamine transport by ApGLNT1 is dependent on external substrate concentration, pH gradient across the membrane, and membrane potential.

2. Material and methods

2.1. ApGLNT1 cDNA cloning

ApGLNT1 (ACYPI001018, LOC100159667) expression construct was generated previously, as described by Price et al. [20]. Briefly, *ApGLNT1* (NM_001246261) full-length coding sequence was amplified from *A. pisum* bacteriocyte cDNA using Phusion proof-reading polymerase (Finnzymes), cloned into NotI and BamHI sites of pcDNA3.1 (Invitrogen) and the sequence was verified using Sanger sequencing [20].

2.2. ApGLNT1 sequence analysis

Transmembrane topology of ApGLNT1 was predicted using TOPCONS consensus prediction program [25] and visualized using PROTTER version 1.0 [26]. Conserved amino acid residues were identified by Clustal Omega multiple sequence alignment of ApGLNT1 and closely related orthologs from other taxa that had previously been identified through phylogenetic reconstruction [19,22]. ApGLNT1 sequence orthologs included mammalian proton-dependent amino acid transporters (PATs) from *Homo sapiens* (hPAT 1–4) and *Mus musculus* (mPAT 1–4) and insect transporters from *Apis mellifera* (XP_394217), *Bemisia tabaci* (Btab_AAAP9), *Diceroprocta semicincta* (Dsem_AAAP8 and Dsem_AAAP8), *Drosophila melanogaster* (CG6327), *Pediculus humanus* (PHUM540430), *Planococcus citri* (Pcit_AAAP7) and *Tribolium castaneum* (XP_969657) [19,22]. All amino acid transporter accession numbers are listed in the electronic supplementary material, Table S1 and all nucleotide sequences are available in electronic supplementary material, dataset S1.

2.3. Expression of ApGLNT1 in Xenopus oocytes

X. laevis oocytes were purchased from EcoCyte Bioscience. Capped *ApGLNT1* copy RNA (cRNA) was generated using T7 mMESSAGE mMACHINE kits (Ambion) and was polyadenylated using the poly(A) tailing kit (Ambion). Oocytes were injected with 23 nl of water (sham) or 23 nL of water containing 23 ng of *ApGLNT1* cRNA (from here on called ApGLNT1-oocytes). Oocytes were incubated at 16 °C in Barth's saline [in mM: 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.3 $CaNO_3$, 0.41 $CaCl_2$, 0.82 $MgSO_4$, 15 HEPES (pH 7.6) and 150 μg/ml ceftazidime] for 1–3 days prior to uptake assay or electrophysiological recording.

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