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Physiology

The cockroach *Blattella germanica* obtains nitrogen from uric acid through a metabolic pathway shared with its bacterial endosymbiontRafael Patiño-Navarrete¹, Maria-Dolors Piulachs², Xavier Belles², Andrés Moya¹, Amparo Latorre¹ and Juli Peretó^{1,3}¹Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, C/Catedràtic José Beltrán n° 2, Paterna 46980, Spain²Institut de Biologia Evolutiva (Consejo Superior de Investigaciones Científicas and Universitat Pompeu Fabra), Passeig Marítim de la Barceloneta n° 37-49, Barcelona 08003, Spain³Departament de Bioquímica i Biologia Molecular, Universitat de València, Burjassot 46100, Spain

Uric acid stored in the fat body of cockroaches is a nitrogen reservoir mobilized in times of scarcity. The discovery of urease in *Blattabacterium cuenoti*, the primary endosymbiont of cockroaches, suggests that the endosymbiont may participate in cockroach nitrogen economy. However, bacterial urease may only be one piece in the entire nitrogen recycling process from insect uric acid. Thus, in addition to the uricolytic pathway to urea, there must be glutamine synthetase assimilating the released ammonia by the urease reaction to enable the stored nitrogen to be metabolically usable. None of the *Blattabacterium* genomes sequenced to date possess genes encoding for those enzymes. To test the host's contribution to the process, we have sequenced and analysed *Blattella germanica* transcriptomes from the fat body. We identified transcripts corresponding to all genes necessary for the synthesis of uric acid and its catabolism to urea, as well as for the synthesis of glutamine, asparagine, proline and glycine, i.e. the amino acids required by the endosymbiont. We also explored the changes in gene expression with different dietary protein levels. It appears that the ability to use uric acid as a nitrogen reservoir emerged in cockroaches after its age-old symbiotic association with bacteria.

1. Introduction

Insect endosymbionts supply their hosts with nutrients needed for their particular lifestyles, mainly essential amino acids or vitamins. Besides, many animals also rely on microbial endosymbionts to recycle their nitrogenous waste products. For instance, in the aphid *Acyrtosiphon pisum*, the ammonia generated in the bacteriocytes (cells containing bacterial endosymbionts) is incorporated into the carbon skeletons of essential amino acids that are generated by *Buchnera aphidicola* [1]. In other insects, like the shield bug, *Parastrachia japonensis*, or the brown planthopper, *Nilaparvata lugens*, endosymbionts enable the host to use uric acid as a nitrogen source during starvation periods [2,3].

It is well known that cockroaches are able to accumulate uric acid when they are fed on a protein-rich diet, and conversely the amount of uric acid stored decreases when they are deprived of proteins [4,5]. Classic observations have suggested that the endosymbiont *Blattabacterium* lies behind these fluctuations. For example, observations show that bacteriocytes are closely associated with uricocytes in the host's fat body, a cell type storing urates [5]. We also know that aposymbiotic individuals of *Blattella germanica* accumulate high amounts of uric acid [6]. The identification of genes encoding for all enzymes of the urea cycle and for urease in the *Blattabacterium* genome [7,8], as well as the results of flux

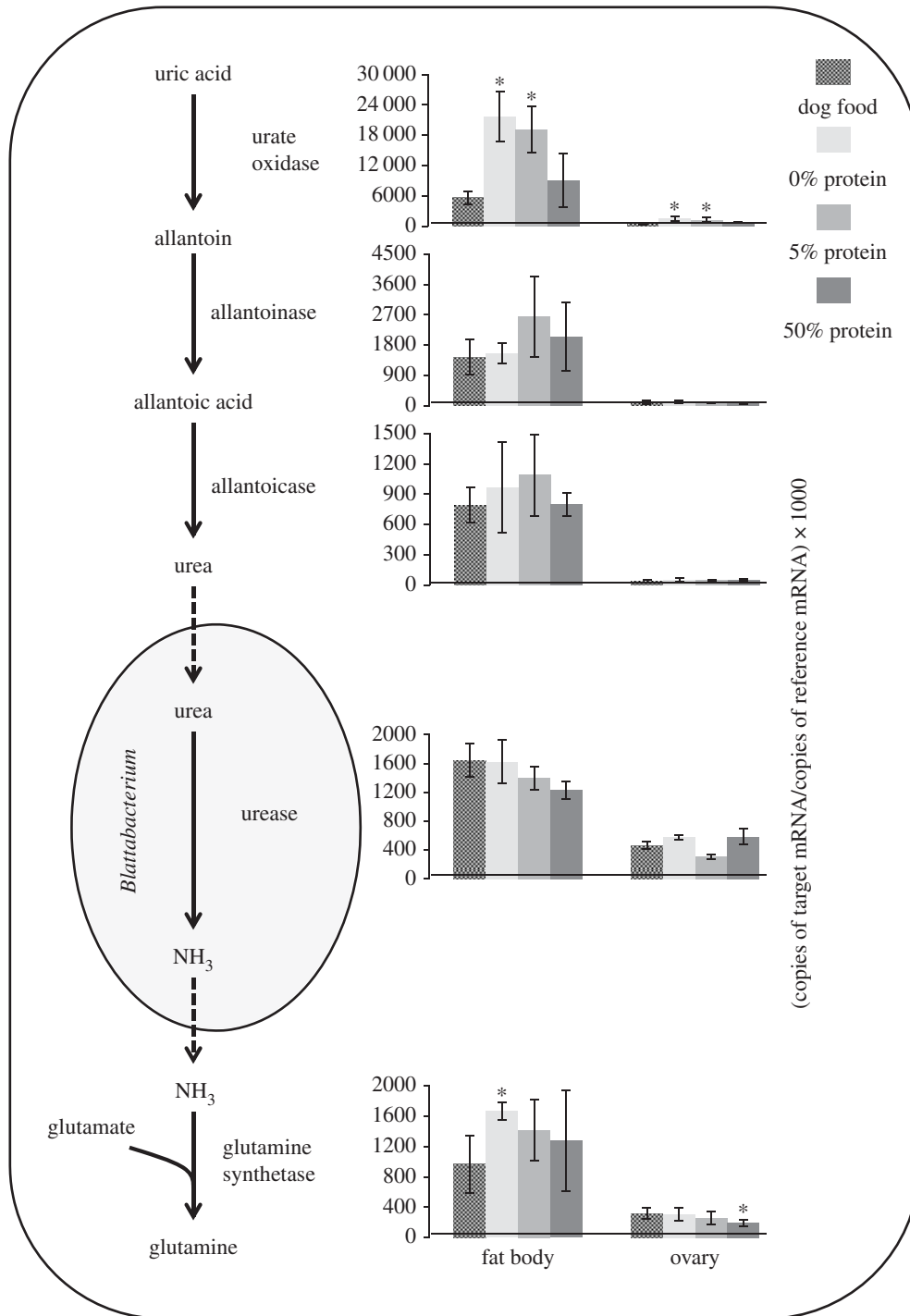


Figure 1. Proposed model for uric acid mobilization. The expression pattern in response to dietary protein levels is expressed beside each gene as copies of mRNA from the target gene per 1000 copies of reference gene (actin 5c and EF-Tu for *Blattella* and *Blattabacterium* transcripts, respectively). The asterisk represent statistically significant differences with respect to control ($p < 0.05$, $n = 3$).

balance analysis (FBA) carried out on the reconstructed metabolic networks of *Blattabacterium* strains from the cockroaches *B. germanica* and *Periplaneta americana* support the key role of the endosymbiont in cockroach nitrogen metabolism [9]. The analysis of six further strains reinforced this hypothesis as the genes for urease and most of the genes of the urea cycle are part of the core of the *Blattabacterium* pangenome [10,11]. Genome-scale metabolic modelling is consistent with these ideas and also shows that *Blattabacterium* is auxotrophic for several non-essential amino acids, including glutamine [9].

Based on these studies, a model was proposed where the uric acid accumulated in the cockroach fat body was used as a nitrogen reservoir, to be mobilized in periods of scarcity [7,9]. This model requires a host uricolytic pathway (i.e. urate

oxidase, allantoinase and allantoicase) and also the supply of non-essential amino acids to the endosymbiont. Despite the presence of many of these enzymes among Bacteroidetes [8], none of the *Blattabacterium* genomes sequenced so far contains the necessary genes [10,11]. However, urate oxidase activity was detected in some tissues of the cockroaches *Leucophaea maderae* [12] and *P. americana* [13]. In the context of this metabolic model, we have also proposed the action of membrane facilitators for urea and glutamine coded in the *Blattabacterium* genome, i.e. *glpF* and *glpP* genes, respectively [7].

This work investigates the presence of transcripts for enzymes involved in nitrogen metabolism in the transcriptome of three *B. germanica* tissues. Two tissue types harbour *Blattabacterium*: the fat body where the bacterium is massively

Table 1. Presence (+) or absence (–) of transcripts related to non-essential amino acid biosynthesis in the three tissue libraries (fat body, ovary and epidermis) of *B. germanica*. (All transcripts, even those represented by a single read, were considered. See the electronic supplementary material, table S3, for accession numbers and best BLAST hits. EC, enzyme commission number.)

gene	EC	fat body	ovary	epidermis
asparagine biosynthesis				
aspartate aminotransferase (mitochondrial-like and cytoplasmic)	2.6.1.1	+	+	+
asparagine synthetase	6.3.5.4	+	+	–
glutamine biosynthesis				
glutamate dehydrogenase	1.4.1.3	+	+	+
glutamine synthetase	6.3.1.2	+	+	+
proline biosynthesis				
glutamate-semialdehyde dehydrogenase	2.7.2.11	+	–	–
ornithine- δ -transaminase	2.6.1.3	+	+	–
pyrroline-5-carboxylate reductase (isozymes P5CR and P5CR2)	1.5.1.2	+	+	–
glycine biosynthesis				
phosphoglycerate dehydrogenase	1.1.1.95	+	+	+
phosphoserine transaminase	2.6.1.52	+	+	–
phosphoserine phosphatase	3.1.3.3	+	–	–
serine hydroxymethyltransferase	2.1.2.1	+	+	+

present, and the ovary, where only a small population of bacteria is present. The third tissue type (the epidermis, including cuticle layers) is a *Blattabacterium*-free tissue. We have also explored how genes involved in uric acid metabolism respond to dietary nitrogen levels. Additionally, we have been able to find the transcripts for the synthesis of the non-essential amino acids required by *Blattabacterium* metabolism.

2. Material and methods

Blattabacterium germanica specimens were obtained from a population reared at the facilities of the Institut de Biologia Evolutiva (CSIC-UPF) in Barcelona, Spain. RNA extraction and cDNA synthesis were performed using standard procedures. Each transcriptome library was sequenced on the 454-Flx platform, assembled and annotated (see the electronic supplementary material).

The relative expression of genes involved in uric acid metabolism was measured in animals fed on different experimental diets with different protein content (0, 5 and 50%), using animals fed on dog food (25% of protein content) as a control (see the electronic supplementary material). Results are represented as copies of target mRNA against the corresponding reference gene (actin 5c and elongation factor EF-Tu in the case of host and endosymbiont transcripts, respectively). Statistical analyses were run with REST [14] (see the electronic supplementary material for further details).

3. Results and discussion

(a) Uric acid metabolism is shared between host and endosymbiont

The nitrogen recycling process in cockroaches involves the degradation of uric acid to urea, and the later degradation of this metabolite by a *Blattabacterium* urease, generating ammonia and CO₂. It has been postulated that endosymbiont-released ammonia would be used by a host-encoded glutamine synthetase to produce glutamine, thus incorporating nitrogen from uric acid to metabolism [7,9]. The expression of the genes for

uricolytic enzymes was detected in the library obtained from the fat body (figure 1). Conversely, only urate oxidase and allantoinase transcripts were detected in the ovary library, whereas none of these genes were expressed in the epidermis library. With the expression of genes for all uricolytic enzymes and glutamine synthetase, the pathway postulated for uric acid recycling would be possible in the fat body (figure 1). On the strength of these results, we can propose that *B. germanica* possesses a nitrogen recycling system similar to the one observed in *P. japonensis* [3] or in *N. lugens* [2], albeit differing greatly with these systems where the uricolytic activities are supplied by the symbionts: in *B. germanica*, the pathway is chimeric with participation of enzymes from the host and the symbiont.

(b) Host metabolism complements non-essential amino acid auxotrophies of *Blattabacterium*

Glutamine is not the only non-essential amino acid required by the endosymbiont metabolism. The FBA of the genome-scale metabolic network of *B. germanica*–*Blattabacterium* would suggest that *Blattabacterium* is also auxotrophic for L-Asn, Gly and L-Pro [9]. Transcripts from all necessary genes for the synthesis of these amino acids were identified in the fat body library, but not in the ovary or the epidermis libraries (table 1). Interestingly, some of these non-essential amino acids are among the most abundant free amino acids in cockroach haemolymph, as measured in *Blaberus discoidalis* [15] and in *P. americana* [16], L-Pro and Gly being the most abundant in both species. The loss of the ability to synthesize non-essential amino acids seems to be a common feature in other insect endosymbionts such as *Buchnera* [17] or *Blochmannia* [18], which are endosymbionts of aphids and *Camponotus* ants, respectively. In aphids, like cockroaches, these non-essential amino acids are also among the most abundant in the haemolymph [1], and their availability in host tissues renders maintenance of biosynthetic pathways for them unnecessary in the endosymbiont. *Blattabacterium germanica*

might use the amino acid supply to control the metabolic behaviour or growth rate of *Blattabacterium*, like the control that the aphid *A. pisum* exerts on the essential amino acid metabolism of *Buchnera* by modulating the supply of metabolic precursors [19,20]. This sort of control over the symbiotic population through amino acid supply has also been observed in plant hosts when controlling their nitrogen-fixing bacteria [21].

(c) Dietary nitrogen levels affect gene expression

Once we had confirmed that the fat body of *B. germanica* expresses genes involved in uric acid production and degradation, we measured the expression of these genes in the fat body and ovary in response to dietary nitrogen levels. Urate oxidase gene expression increased significantly in both tissues of animals fed on a low-protein diet (figure 1). The other gene showing a significant variation in expression is the one for glutamine synthetase, which is over-expressed in the fat body of animals fed on a non-protein diet, and downregulated in the ovary of those animals fed on a high-protein diet (figure 1). None of the other genes showed significant increases in expression, suggesting that the uricolytic pathway is expressed in a constitutive manner and other levels of flux regulation must exist.

Cockroaches accumulate uric acid in the fat body, especially specimens fed on protein-rich diets [5]. The amount of uric acid accumulated in these animals decreases dramatically when they are shifted to a low-protein diet [22]. Both observations suggest that uric acid is actually a reservoir of nitrogen, ready to be mobilized in periods of scarcity. Our observations on the increased gene expression for urate oxidase and glutamine synthetase in animals deprived of a dietary nitrogen source are consistent with this proposal.

We can conclude, thus, that after the symbiotic association between the ancestors of cockroaches and *Blattabacterium*, their metabolic networks merged and transformed a nitrogen waste product in insects, such as uric acid, into a metabolically useful source of nitrogen.

Data accessibility. Available in the electronic supplementary material, table S3.

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