Cooperation between symbiotic partners through protein trafficking

Mariana Galvão Ferrarini, Mélanie Ribeiro Lopes, Rita Rebollo

Cooperation between symbiotic partners through protein trafficking

Mariana Galvão Ferrarini[1#], Mélanie Ribeiro-Lopes[1#] and Rita Rebollo[1#]

1 INRAE, INSA Lyon, BF2I, UMR203, 69621 Villeurbanne, France

corresponding authors

Symbiosis can act as a major driver of evolution, as symbionts' genetic resources can enhance host resistance to stress and expand their ecological niches (1). This is particularly true for endosymbionts, *i.e.* symbionts that live within the body or cells of their host, as the intimate contact between partners favours molecule exchanges. However, apart from metabolites, the extent of macromolecule trafficking between endosymbiotic partners remains underexplored. In the current issue of PNAS, Ling et al. (2) provide novel insights into this phenomenon. They provide evidence that not only endosymbionts can shuttle entire proteins across several membranes, but proteins from different organisms can cooperate *in vivo* to increase host adaptability to environmental changes.

Endosymbiosis is widespread in nature and particularly prevalent among insect species. Notably, insects thriving on nutritionally poor or unbalanced diets (e.g. plant sap, seeds, blood), have repeatedly established obligate endosymbioses with bacteria that complement their diet with nutrients lacking in their habitat (1). Endosymbionts are often housed within specialized cells of the host named bacteriocytes (3). Due to a relaxed evolutionary pressure within these cells, the bottleneck effect from vertical transmission, and the absence of recombination with free-living relatives, these obligate endosymbionts experience significant genome reduction, including the loss of virulence genes along with genes redundant with the host pathways or unnecessary in the new habitat (4). Genomic erosion is also promoted by the loss of the endosymbiotic DNA repair machinery, which accelerates the accumulation of mutations. In the textbook example of obligate endosymbiosis, the bacteriocyte-bound symbionts of aphids, *Buchnera aphidicola,* have lost most ancestral genes and have specialized in the production of amino acids required by aphids.

While reducing the endosymbiotic genome leads to a cost-effective metabolic interaction between host and bacteria, the massive genomic erosion can also lead to negative effects on both symbiotic partners, including the restriction of ecological niches and the ultimate risk of extinction in case of increased environmental pressure (4). *Buchnera*, along with other reduced-genome obligate endosymbionts, lack the plasticity to adapt to environmental changes, which makes them particularly vulnerable to biotic and abiotic stresses and can compromise the host's

survival. Heat tolerance, for instance, varies drastically among aphid species and was shown to be associated in part with *Buchnera* genetic features (5). Indeed, a single mutation in the homopolymer promoter of Inclusion body-associated protein A (*IbpA*), a prokaryotic small heat shock protein gene, impairs *Buchnera ibpA* expression and is linked to reduced aphid survival upon heat stress (5). Replacement of *ibpA*-deficient *Buchnera* with wild-type *Buchnera* rescues the host from heat-shock events (6).

The deleterious effects of the irreversible and extensive endosymbiont gene loss can be countered by the replacement of the primary endosymbiont or the colonization by a facultative symbiont that further expands the capabilities of the symbiotic system (4). In some cases, in particular when genes involved in essential nutrient biosynthesis are lost, facultative symbionts can even become obligate, leading to co-obligate symbiosis (3). The interaction between insects and their obligate endosymbionts is often sensitive to heat stress, potentially explaining the widespread examples of facultative endosymbionts associated with heat tolerance (7). *Serratia symbiotica* has been shown to increase aphid thermotolerance and field studies have shown that the incidence of *Serratia* increases in warmer climates compared to colder ones, even at the same site of sampling, suggesting that *Serratia* protection to heat-stress benefits the host and might therefore favour *Serratia* prevalence (8). In pea aphids, previous studies suggested that *Serratia* protects the host by releasing metabolites shielding *Buchnera* upon heat stress (9). Yet, aphids lacking *Serratia* have an increased prevalence of the *Buchnera* mutated *ibpA* gene both in the field and in laboratory strains (5, 10), suggesting further compensation mechanisms between the endosymbionts.

Ling et al. (2) showcase a remarkable cooperation between *Buchnera* and *Serratia* under heat stress and provide a mechanistic explanation for the *Serratia*-associated thermotolerance of infected aphids, whereby gene complementation between the two endosymbionts restores an incomplete DNA mismatch repair (MMR) system in *Buchnera*. Indeed, the MMR system safeguards the integrity of the genome, repairing DNA errors arising through replication, thanks to the recruitment of different proteins, including MutS, MutL and MutH (11). MutS homodimer recognizes mismatched bases and recruits the MutL homodimer that activates the endonuclease MutH, which is absent in *Buchnera* and present in the *Serratia* genome. MutH nicks the DNA strand near the mismatch allowing UvrD helicase and an exonuclease to degrade the DNA. Finally, the DNA gap is repaired by a DNA polymerase followed by DNA ligation (11). Ling et al. (2) provide microscopy and mass spectrometry evidence for the translocation of the *Serratia* MMR protein MutH outside of *Serratia* and the sheath cells in which they are located (*i.e*. small cells that surround bacteriocytes and can harbor facultative

symbionts), into the aphid bacteriocytes and further into *Buchnera* cells (Fig. 1). *Serratia* MutH appears to complement *Buchnera* MutL and MutS proteins to form an active MMR. This is proposed to slow the accumulation of mutations, including the detrimental *Buchnera ibpA* allele prevalent in *Serratia*-free aphids.

While metabolic complementation (the exchange of metabolites) between species has been extensively studied (12), the exchange of entire proteins, particularly across multiple membranes, remains poorly understood. Existing literature highlights instances where hosts provide proteins missing from the genomes of endosymbionts (Fig. 1) [(13–17), reviewed in detail by (18)]; however, the novelty presented in Ling et al. (2), is that other endosymbionts can also be a source of protein exchange and adaptation. Moreover, *Serratia* MMR protein MutH was not the only *Serratia*-encoded protein found inside *Buchnera* cells, and other proteins related to nutrient metabolism, gene regulation and stress response were also detected, highlighting the fact that the extent of protein exchange could be much larger than anticipated.

A key unanswered question is how these proteins are trafficked across multiple membrane layers and remain intact, especially when endosymbionts are physically compartmentalized in different cells, as seen with *Buchnera* in bacteriocytes, and *Serratia* in sheath cells and/or other bacteriocytes (distinct from the ones harboring *Buchnera*) (Fig. 1). Outside the insect world, two model systems from unicellular protists, the amoeba *Paulinella chromatophora* and the kinetoplastid *Angomonas deanei* were shown to bear targeting signals for the correct addressing of host nuclear proteins back to their endosymbionts, similarly to what is seen with organelles (15, 16). Even though the mechanism remains unclear, these proteins are thought to be transported either through endoplasmic reticulum vesicles or outer membrane channels. Within insects, host-encoded proteins were shown to be shuttled into endosymbionts in the aphid-*Buchnera* (14), the tripartite nested mealybug (13), the cereal weevil-*Sodalis* (19), and the red palm weevil-*Nardonella* (17) systems. In the case of aphids, each *Buchnera* cell within bacteriocytes is individually surrounded by a host-derived symbiosomal membrane, and the molecular basis of transport across such membrane remains largely unresolved. The mealybug (*Planococcus citri*) system is even more complex, as there are two endosymbionts, and one (*Moranella endobia*) resides in the cytoplasm of the other (*Tremblaya princeps*). Proteins from the host are transferred to the innermost endosymbiont, *Moranella*, and do not accumulate in the cytoplasm of *Tremblaya*, to create a peptidoglycan layer around *Moranella* cells (13). Although the mechanism of protein trafficking remains to be elucidated, the authors also pointed out that it could be the mRNA rather than the protein that is trafficked to the endosymbiont cells instead. Ling et al. (2), also suggest that aphid bacteriocytes have increased expression of

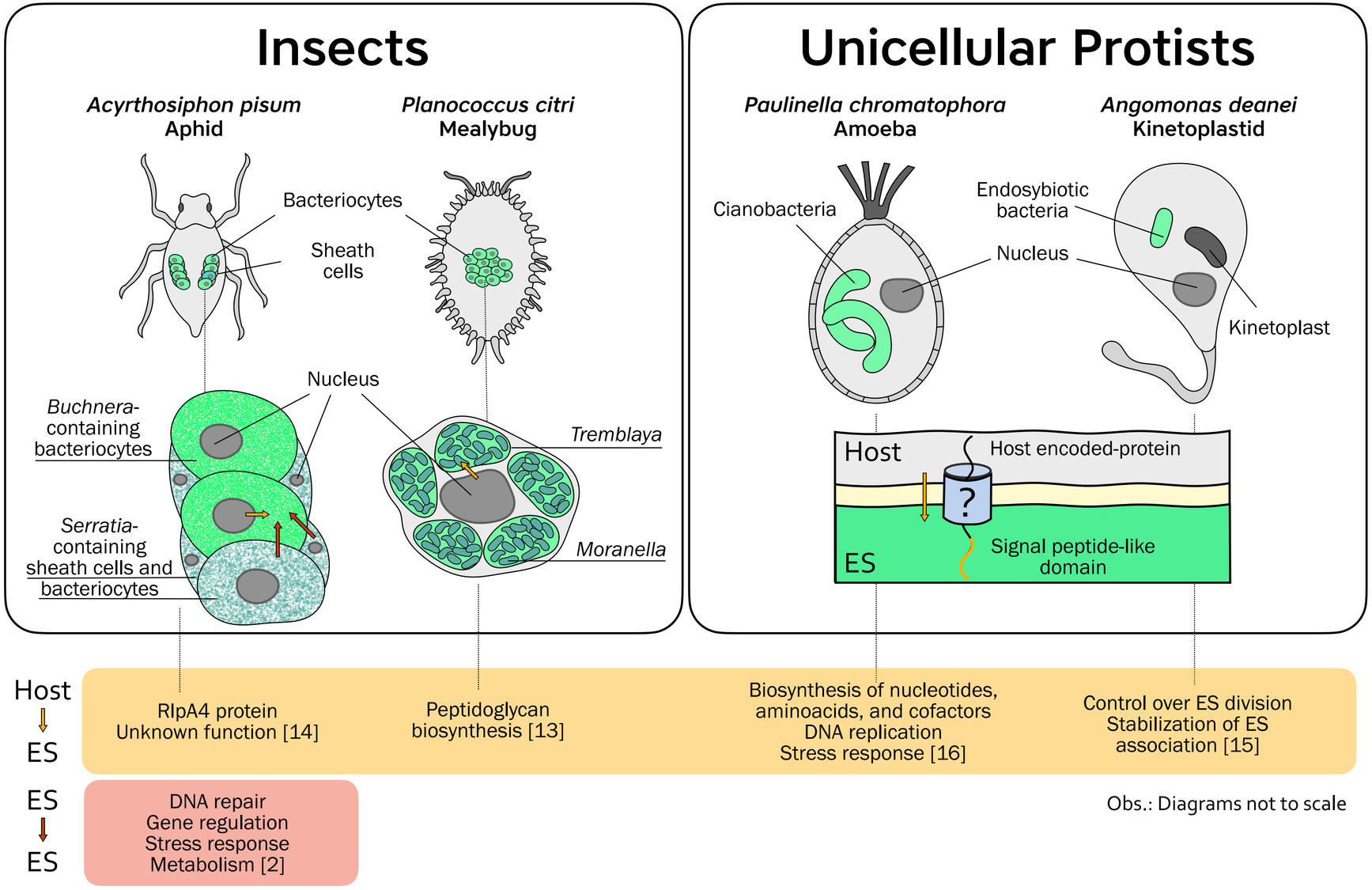
genes involved in vesicle synthesis and transport compared to other aphid tissues, and *Serratia* bears genes associated with outer membrane vesicle formation, further supporting the hypothesis of vesicle trafficking. Whatever the mechanism, it is clear that organisms can exchange such macromolecules, directly affecting their adaptability traits.

Unfortunately, identifying the process that governs exchanges between symbiotic partners is hampered by the fact that the majority of insect endosymbionts, including *Buchnera* and most *Serratia symbiotica* strains, are not culturable *in vitro* due to extensive genome erosion, preventing functional genetic assays (20). Finally, Ling et al. (2) and others highlight how the beneficial effects brought on by facultative endosymbionts are contingent on the environment and might remain cryptic under normal conditions (4, 7), stressing the need for multi-context dependent studies.

1. N. A. Moran, Symbiosis. *Curr. Biol. CB* **16**, R866-871 (2006).
2. X. Ling, *et al.*, A complete DNA repair system assembled by two endosymbionts restores heat tolerance of insect host.
3. S. Sudakaran, C. Kost, M. Kaltenpoth, Symbiont Acquisition and Replacement as a Source of Ecological Innovation. *Trends Microbiol.* **25**, 375–390 (2017).
4. G. M. Bennett, N. A. Moran, Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole. *Proc. Natl. Acad. Sci.* **112**, 10169–10176 (2015).
5. H. E. Dunbar, A. C. C. Wilson, N. R. Ferguson, N. A. Moran, Aphid Thermal Tolerance Is Governed by a Point Mutation in Bacterial Symbionts. *PLOS Biol.* **5**, e96 (2007).
6. N. A. Moran, Y. Yun, Experimental replacement of an obligate insect symbiont. *Proc. Natl. Acad. Sci.* **112**, 2093–2096 (2015).
7. C. Corbin, E. R. Heyworth, J. Ferrari, G. D. D. Hurst, Heritable symbionts in a world of varying temperature. *Heredity* **118**, 10–20 (2017).
8. C. B. Montllor, A. Maxmen, A. H. Purcell, Facultative bacterial endosymbionts benefit pea aphids Acyrthosiphon pisum under heat stress. *Ecol. Entomol.* **27**, 189–195 (2002).
9. G. Burke, O. Fiehn, N. Moran, Effects of facultative symbionts and heat stress on the metabolome of pea aphids. *ISME J.* **4**, 242–252 (2010).
10. G. R. Burke, H. J. McLaughlin, J.-C. Simon, N. A. Moran, Dynamics of a Recurrent Buchnera Mutation That Affects Thermal Tolerance of Pea Aphid Hosts. *Genetics* **186**, 367–372 (2010).
11. C. D. Putnam, Evolution of the methyl directed mismatch repair system in Escherichia coli. *DNA Repair* **38**, 32–41 (2016).
12. A. K. Hansen, N. A. Moran, The impact of microbial symbionts on host plant utilization by herbivorous insects. *Mol. Ecol.* **23**, 1473–1496 (2014).
13. D. C. Bublitz, *et al.*, Peptidoglycan Production by an Insect-Bacterial Mosaic. *Cell* **179**, 703-712.e7 (2019).
14. A. Nakabachi, K. Ishida, Y. Hongoh, M. Ohkuma, S. Miyagishima, Aphid gene of bacterial origin encodes a protein transported to an obligate endosymbiont. *Curr. Biol.* **24**, R640–R641 (2014).
15. J. Morales, *et al.*, Host-symbiont interactions in Angomonas deanei include the evolution of a host-derived dynamin ring around the endosymbiont division site. *Curr. Biol.* **33**, 28-40.e7 (2023).
16. A. Singer, *et al.*, Massive Protein Import into the Early-Evolutionary-Stage Photosynthetic

Organelle of the Amoeba Paulinella chromatophora. *Curr. Biol.* **27**, 2763-2773.e5 (2017).
17. Y. Huang, Z.-F. Feng, F. Li, Y.-M. Hou, Host-Encoded Aminotransferase Import into the Endosymbiotic Bacteria Nardonella of Red Palm Weevil. *Insects* **15**, 35 (2024).
18. M. E. S. Sørensen, M. L. Stiller, L. Kröninger, E. C. M. Nowack, Protein import into bacterial endosymbionts and evolving organelles. *FEBS J.* **n/a**.
19. F. H. Login, *et al.*, Antimicrobial Peptides Keep Insect Endosymbionts Under Control. *Science* **334**, 362–365 (2011).
20. F. Masson, B. Lemaitre, Growing Ungrowable Bacteria: Overview and Perspectives on Insect Symbiont Culturability. *Microbiol. Mol. Biol. Rev. MMBR* **84**, e00089-20 (2020).

Figure 1: Central biological functions impacted by protein trafficking from host to endosymbiont (ES) (in yellow) in insects and unicellular protists, and the first case of protein shuttling between aphid endosymbionts (in red) as evidenced by Ling et al (2).


Insects
Acyrthosiphon pisum
Aphid
Planococcus citri
Mealybug
Bacteriocytes
Sheath cells
Nucleus
Buchnera-containing bacteriocytes
Serratia-containing sheath cells and bacteriocytes
Tremblaya
Moranella
Unicellular Protists
Paulinella chromatophora
Amoeba
Angomonas deanei
Kinetoplastid
Cianobacteria
Nucleus
Endosybiotic bacteria
Kinetoplast
Host
Host encoded-protein
?
ES
Signal peptide-like domain
Host
ES
RIpA4 protein
Unknown function [14]
Peptidoglycan biosynthesis [13]
Biosynthesis of nucleotides, aminoacids, and cofactors
DNA replication
Stress response [16]
Control over ES division
Stabilization of ES association [15]
ES
ES
DNA repair
Gene regulation
Stress response
Metabolism [2]
Obs.: Diagrams not to scale