# Endosymbiosis: Protein Targeting Further Erodes the Organelle/ Symbiont Distinction

**New work in aphids shows that a nuclear-encoded protein resulting from a horizontal gene transfer is targeted to a bacterial symbiont, further blurring the distinction between organelle and symbiont.**

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Although the endosymbiotic origin of eukaryotic plastids and mitochondrial organelles is now beyond serious question, the actual process by which bacteria transformed into highly integrated and derived organelles remains contentious. Especially in the case of mitochondria, the nature of the partners, which one predominantly drove the process, and for what reason are all still debated [1,2]. This is partly due to the thoroughness of the transformation, as well as the extreme antiquity of the events, both of which erase evidence. For this reason there is considerable interest in observing more recent endosymbiotic partnerships as they unfold: they are not only fascinating in their own right, but if their evolution is similar to that of organelles, then they might provide a glimpse into hypothesized early intermediates in organelle evolution. One stage that has repeatedly been identified as key to this transition (or indeed, to represent *the* boundary between endosymbiont and organelle) is the horizontal transfer of genes from the endosymbiont to the host, and the evolution of a protein targeting system to direct the products of these genes back to the nascent organelle [3–6]. The search for horizontally transferred genes in multiple analogous endosymbiotic systems has revealed numerous examples [7–9]. However, except for the noteworthy exception of the plastid-like symbiont of *Paulinella* [4], the cellular localization and function of these gene products are typically unclear. In this issue of *Current Biology*, Nakabachi *et al.* [10] describe an important piece in this puzzle, with their demonstration that an animal genome contains a gene of bacterial origin whose protein product is targeted to an intracellular bacterial symbiont. Using antibodies raised against the predicted protein sequence of an alphaproteobacterium-derived gene in the genome of the pea aphid, *Acyrthosiphon pisum*, Nakabachi *et al.* show that this protein is specifically targeted to the aphid's nutritional gammaproteobacterial endosymbiont, *Buchnera aphidicola* [10]. This result provides evidence that the complex genetic mosaicism predicted in some insects is functionally relevant, and establishes further interesting parallels to organelle biology.

Previous work had established that the genome of pea aphid encodes a handful of genes of both bacterial [7] and fungal [11] origin. The bacterial genes came from two distinct phylogenetic groups. One set of transfers originated from the endosymbiont *Buchnera*, but these genes were inferred to be non-functional because they were pseudogenized and not expressed. The other set of bacterial genes appear to be functional but are phylogenetically related to homologues from alphaproteobacteria, and more specifically to the insect reproductive manipulators *Rickettsia* and *Wolbachia* [7]. The demonstration by Nakabachi and colleagues that the protein product of one of these genes, *rplA4*, is specifically localized to *Buchnera* cells [10] strongly suggests that this protein has acquired some function related to *Buchnera*, rather than some host process unrelated to the symbiosis. What process might this be? It is currently unclear, but RplA has been shown to be a lytic transglycosylase with a role in cell shape and peptidoglycan breakdown in *Pseudomonas aeruginosa* [12]. While the functional details are not known in this case, it is worth considering the inferred function and taxonomic sources of horizontal gene transfers (HGTs) in animals to get a sense of what these results mean in the context of symbiosis generally.

The functional consequences of successful HGTs are not always clear, but for the purposes of this discussion, we can divide them into two broad categories. The first are what might be called 'adaptive transfers', where HGT endows the recipient organisms with new functions they previously did not have. These kinds of transfers are not unique to animals or to endosymbiotic systems, and are especially common in Bacteria, where HGT is thought to play a major role in adaptation to new environments. Although less frequent, cases of adaptive transfers to animals are also emerging, for example the coffee berry borer beetle, where HGT of a bacterial carbohydrate degrading gene seems to have allowed these insect pests to use coffee beans as a food source [13]. Many other HGTs, however, do not seem to provide a novel function to the recipient, but are more easily explained as events that simply preserve a pre-existing function. For example, there may or may not be a benefit when a transferred gene replaces an existing gene, because the event does not appear to impart a completely new function to the recipient. This category of events might be called 'compensatory transfers', and seem to be quite common in endosymbioses that become stable over long periods of time. This process has been hypothesized to have taken place in organelle evolution, where HGT from multiple sources enables gene loss on the organelle genome [2,14–16]. Compensatory transfers have also been described in sap-feeding insects that have developed stable endosymbioses with bacteria. For example, in both psyllids and mealybugs, HGT from various bacterial sources to the insect genome seems to enable the loss of critical nutritional genes on the endosymbiont genomes [8,9]. Although the function of the RplA4 protein in this pea aphid is not clear, present evidence points to it being a compensatory transfer.

Why would compensatory transfers be common in stable endosymbioses? The answer may be related to the massive gene loss experienced by genomes of intracellular symbionts. Some of this loss is doubtless simply due to the transition from free-living bacterium to endosymbiont, which would be expected to render a large number of genes free to be lost without consequence. Some genes, in particular those required for basic

cellular or symbiotic functions, cannot be lost without harmful or fatal results. But these genes can be replaced, and perhaps ever more easily as the interaction networks of the endosymbiont reduce in complexity, thus reducing pressures on proteins to co-evolve. In a sense, when genes are transferred from symbiont or organelle to the host nuclear genome and their proteins targeted back, this is a compensatory change [4,9,14,17]. But other compensatory transfers that affect a symbiont or organelle can also involve genes transferred from unrelated donors [8,9,18]. In the case of organelles the identity of potential donors of transferred genes is not always clear, but in insects gene donors often appear to be pathogens, specifically reproductive manipulators [7–9]. These bacteria skew the number and sex ratio of offspring in infected populations in different ways, and often reside in insect germ line cells. The frequency of transfer from these groups is therefore probably due to simple chance: their cell biology includes infection of the germ line, which provides ample opportunity for gene transfer that can be passed to future generations. The same is true of single-celled eukaryotes (protists), where all newly acquired genes are taken into the germline automatically [17].

As the prevalence of intimate and stable endosymbiotic associations has become more clear, the degree to which host and endosymbiont are integrated has been revealed to be far less discontinuous than previously believed. Accordingly, the characteristics separating 'symbiont' from 'organelle' have become less clear [3,4,19,20]. There is an understandable desire to draw a distinct line between the two for simplicity, but first we must ask, does this line exist? If so, it is best drawn by evolutionary and mechanistic distinctions, not by perceived differences born of tradition, definitions, or historical contingency. Organelles were discovered first, have been studied for decades, and their bacterial origins dominated the discussion about endosymbiosis and evolution for many years. They enjoy a status apart from other biological entities: derived from bacteria, but so different as to be given their own name. But the list of their 'unique' characteristics is shrinking: stable endosymbioses promote extensive genome reduction in the symbiont, HGT from various sources to the host genome to maintain symbiont function, and now the targeting of protein products from host to symbiont has even been found [4,10]. These make clean separation of endosymbiont from organelle more difficult to see, prompting us not to look for the point when a symbiont 'becomes' an organelle, but rather to ask, 'Is there really anything so special about organelles?'

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# Animal Evolution: Looking for the First Nervous System

**The human brain is easily the most baffling bit of biology on the planet. How did the nervous system evolve? What came first: neurons or synaptic proteins? A new paper studying the pancake-shaped *Trichoplax* suggests it was not the neurons.**

**Erik M. Jorgensen**

Something bad must have happened around 542 million years ago: the Ediacaran period, which had seen the rise of complex, unfamiliar looking multicellular marine life forms, ended with an extinction that wiped out most of these creatures. Unfortunately, this event obscured our view on the