Dispatches

Endosymbiosis: Bacteria Sharing the Load

A nested set of bacterial endosymbionts within mealybug cells collectively provides amino acids to their host, but their genomes show that some pathways are distributed between both endosymbionts, while other essential proteins are missing altogether. The possibility that additional functions are shared between partners warrants comparisons with organelles.

Patrick J. Keeling

> Those who have much are often greedy, those who have little always share.
> — Oscar Wilde

Some levels of organisation in biology imply an indivisible nature: cells, organisms, and species as 'minimal units' of functioning diversity can influence our thinking, but the more we learn about the interactions between such entities, the more complex and difficult to defend their definitions become. At the cellular level, our understanding of the interactions between endosymbionts and organelles is transforming any black-and-white picture of these 'species' or 'organisms' into a spectrum of grey areas. In this issue of *Current Biology*, McCutcheon and von Dohlen [1] describe a complex system of molecular interactions between endosymbionts within endosymbionts, and in doing so add another grey area to any strict division between the organisms that make up this remarkable whole.

The bacterial endosymbionts found within insects that specialize on low-nutrient food sources such as plant saps have long been known to display complex adaptations affecting both hosts and endosymbionts [2–4]. One emerging theme is the role of endosymbionts in the production of essential amino acids, which are to varying degrees poorly supplied by sap. Initially, the genomes of bacterial endosymbionts in relatively simple systems (e.g., a long-term symbiosis with a single bacterium) were found to contain a complete set of pathways required to provide the missing amino acids to the animal [5]. A new twist was added with the recognition that some insects contained two distinct bacterial endosymbionts [6], and when their genomes were examined a simple division of labour was discovered: their genomes encoded complete pathways for amino acid synthesis, but in this case each bacteria only synthesized a subset of the required amino acids, so that only together was the complete set available to the animal [6].

The mealybug endosymbiosis takes this further in several directions. Mealybugs have two bacterial endosymbionts, but in this case one lives inside the other in a rare bacterial–bacterial endosymbiosis [7]. By sequencing the complete genomes of both endosymbionts, McCutcheon and von Dohlen [1] have shown that they too have divided the labour of producing essential amino acids, but that some pathways are incomplete in both genomes, and only collectively can the complete pathway be reconstructed. The implication is that intermediates are shuttled between endosymbionts, ultimately resulting in the finished amino acid used by both endosymbionts and their animal host.

This degree of functional integration may be unusual for endosymbionts, at least as far as we now know, but parallels are known from the equally strange world of organelles. Heme biosynthesis is a good example. In many eukaryotes this pathway spans the cytosol and mitochondrion, but in the apicomplexan parasites, which also have a non-photosynthetic plastid, phylogenetic analysis and direct localization of heme biosynthetic enzymes has revealed that the pathway has been partitioned between the mitochondrion, the plastid, and perhaps the cytosol [8–11]. All eukaryotes have the potential to partition pathways between compartments, and in some cases additional layers of endosymbiosis have built a great deal of complexity involving not just three, but four, five, or even six semi-independent compartments (e.g., see Figure 1) [12]. The apicomplexan heme case shows how the direct localization of proteins is necessary to reveal the true complexity of a pathway's winding route through the cell, so in evermore complex systems we might expect to find a concomitant increase in the subdivision of metabolic functions.

Other characteristics of the mealybug endosymbionts also beg comparison to organelles. Superficially, at 139 kbp *Tremblaya* contains the smallest recorded non-organelle genome, falling well within the size range of both plastids and mitochondria (interestingly, the other endosymbiont, *Moranella*, lives inside *Tremblaya* but has a genome about four times larger, a situation unique among endosymbionts). More important is the gene content of *Tremblaya*, which was found to lack a number of genes generally essential to gene expression, and which have been retained in other even heavily reduced endosymbionts [13]. The authors propose that these missing proteins might be supplied by *Moranella*, perhaps passively though lysis [1], but also raise the possibility that *Tremblaya* has not left such essential functions to chance, and that they are specifically targeted to it, either from *Moranella* or from their mealybug host.

Protein-targeting would add a whole new dimension to these interactions, since this step is the most commonly cited point at which an 'endosymbiont' becomes an 'organelle' [14]. Beyond what terminology we use, this process has deep practical implications for both partners in any association, because it initiates a ratchet-like process of genetic integration. Establishing a system that allows the specific targeting of even a single protein is complicated and therefore likely rare [15], but once such a system is in place, the subsequent transfer and targeting of other proteins becomes substantially easier, opening a floodgate of potential gene transfers, each increasing the integration of the partners at the molecular level. All

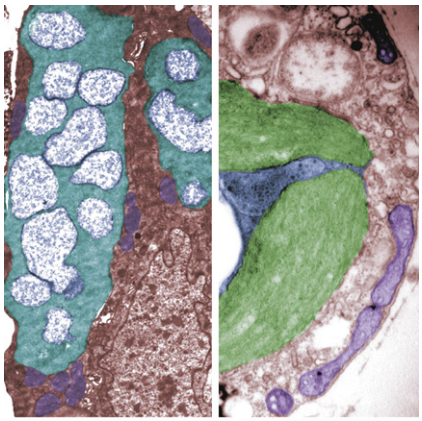

Figure 1. Comparing complex systems of 'endosymbionts' and 'organelles'.

Two false-colour transmission electron micrographs of complex associations involving four partners. To the left is a mealybug cell (red) inside of which lives mitochondria (purple), and *Tremblaya* (turquoise), inside of which lives *Moranella* (blue). To the right is the chlorarachniophyte *Lotharella globosa*, an amoeba (red) that has living within it mitochondria (purple), and a green algal endosymbiont (blue), which itself contains plastids derived from cyanobacteria (green). (Photos courtesy of C.D. von Dohlen and Y. Hirakawa.)

this begs the question, is *Tremblaya* an endosymbiont or an organelle? The answer depends on your definition of these words, but looking for the genes that encode these missing proteins is surely of great interest.

The grey area occupied by organisms like these raises another more practical point, which is the unfortunate drift that is separating research on endosymbionts and organelles. Not very long ago these fields were closely knit, but since both burgeoned into large areas in their own right, comparative analyses are increasingly difficult to pull together, and accordingly scarce.

A considerable collection of data exists regarding mutation profiles, host interactions, genome structure evolution, and so forth, but the similarities and differences between what we call endosymbionts and what we call organelles are under-explored. Given the great overlap in the conditions under which they evolve, and the fantastic parallel examples that are available (especially when intracellular parasites are thrown into this mix), we are not taking full advantage of an opportunity to see whether or not there is a molecular difference between being an endosymbiont and being an organelle.

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Metabolism: What Causes the Gut's Circadian Instincts?

What mechanisms control circadian rhythms in the gastrointestinal tract and how does this impact nutrient metabolism? The deadenylase and leucine zipper protein Nocturnin is now shown to play a central role.

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For centuries, circadian biology has been recognized as a critical physiological adaptation to the light–dark cycle caused by the earth's rotation [1–5]. In today's world, circadian rhythms have their most acute impact when we travel across multiple time zones within a single day. Generally, most of us experience profound difficulty sleeping and maintaining routine physical performance for at least a few days as our bodies acclimate to photic stimuli and other circadian entrainers in the new environment. The molecular mechanisms underlying this phenomenon are conserved throughout the phylogenetic tree [1]. In mammals, a heterodimer of two transcription factors positively controls the ~24 hr circadian oscillatory period. This heterodimer is composed of the BMAL1 (brain and muscle ARNT-like 1) protein in combination with either its Clock (circadian locomotor output cycles kaput) or NPAS2 (neural period/ARNT/simpleminded 2) partner [1], which together bind to promoter elements in target genes and activate transcription. These targets include genes encoding Cry (cryptochrome) and Per (period) proteins which also heterodimerize to