The fate of obligate endosymbionts: reduction, integration, or extinction

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Whether mitochondria and plastids originated by endosymbiosis is no longer questioned, but we still do not understand the actual process of integration. Other, younger endosymbiotic systems are, however, relatively common. Traditionally, it was not clear whether these systems could be directly and informatively compared to organelles because they appear sufficiently different. Surprisingly, new data from both organelles and endosymbiotic bacteria are changing this view. As more commonalities are described, the processes underlaying these associations appear to be not so different after all. New models for endosymbiotic associations emphasize the importance of transient stages, conflict more than cooperation, and population genetics forces that lead to genome reduction, which in turn restricts most endosymbionts to one of a few possible evolutionary pathways, commonly ending with extinction.

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Evolutionary pathways to obligate endosymbiosis

If we could go back in time and observe the origin of mitochondria and plastids, would there be a time when we would call these not organelles but symbiotic bacteria? The answer is surely yes, and indeed this long-debated conclusion is no longer seriously questioned [1–3]. But this certainty belies how much we still don't know about the actual process of organelle origins at a mechanistic level: simply saying 'they originated by endosymbiosis' is a comforting certainty, but one that tells us little or nothing about exactly what events led to this transition, the order of those events, or their timing. The extreme

age of both mitochondria and plastids means data that might definitively address these points are sparse, and in this vacuum debate has thrived [4–9,10•,11].

But before we start, we first need to clarify some terminology (see Box 1), including also how we interpret the term 'symbiosis'. We consider symbiosis to be a continuum of interactions that is highly context-dependent with two or more viewpoints represented by the host and symbiont(s). The particular symbiotic partners, exact timing, environmental conditions, and other factors can influence whether an organism acts as a mutualist, commensal, or pathogen. Distinguishing between these categories, although useful in well-defined simple systems, is not always possible and in our view potentially even misleading. Contexts change over time and assigning firm roles in the short term can give a false impression of what to expect over longer periods of evolutionary time. Symbioses may seem idyllic on the first sight, but most often we should think of them as continuously shifting power struggles [12–14].

The relationship can be dominated by either the symbiont or the host [12–14] and, perhaps counter-intuitively, the more dependent partner must take control. Where the symbiont drives the relationship (Figure 1a), we see ancient lineages of 'symbiotic specialists' that are dependent on their host, but host-specificity can be short-lived. The specialist lineage can persist through long periods of evolutionary time (e.g. *Rickettsiales* and *Holosporales*), but by changing host-associations relatively frequently its distribution is complex and there is little selection for elaborate adaptations to any specific host (like genetic integration—see below). Over time, its members pair-down cellular and metabolic functions, resulting in highly compact genomes packed with genes for survival in eukaryotic hosts (Figure 2).

In contrast, if the host becomes dependent on the endosymbiont, it will dominate the relationship and the outcome is very different (Figure 1b). The symbiont genome will slowly erode due to population genetics processes driving genome reduction. Reduction leads to co-dependence with positive and negative aspects for both partners, a situation referred to as an **'evolutionary rabbit hole'** [14]. The host remains dependent on the endosymbiont, so extreme endosymbiotic reduction leads the host to one of two main pathways to avoid extinction. On one hand, the host may simply replace the endosymbiont with a 'fresh' one from the

Box 1 Symbiosis terminology

The symbiosis field is a fascinating assemblage of researchers from different disciplines studying different model organisms. This diversity is a much-needed spark for new ideas, but it also leads to a 'Babylonian confusion of tongues' when identical terms are used to describe non-identical things. Since we are addressing this diverse community, we wish to clarify the four most confounding terms in this box.

Perhaps the most troublesome terms are **primary and secondary symbiosis**. These terms were introduced to the animal symbiosis research by Paul Buchner [55] who used the term primary symbiont to describe an essential (and putatively ancient) intracellular symbiont that is needed by its host for survival and reproduction (for example *Buchnera* symbionts in aphids). He also recognized the presence of additional morphologically diverse symbionts and described them as 'secondary symbionts' based on the fact that they are not always present and likely not required for the host survival. However, the same terms have completely different meanings in protistology and organelle research, where primary endosymbiosis is defined as the origin of an endosymbiotic organelle from the direct uptake of a bacterium, such as mitochondria and the plastids in Archaeaplastida [35•,36]. Secondary symbiosis is an additional layer of endosymbiosis, where a single-celled eukaryote engulfs another photosynthetic eukaryote, and keeps its 'primary' plastid (for example, euglenid and chlorarachniophyte algae have plastids they acquired from eating green algae with primary plastids) [19].

To prevent the primary versus secondary confusion, insect symbiosis researchers graciously embraced different terms—**obligate** and **facultative symbiosis** (the latter also called 'guest' or 'accessory' symbionts), only to encounter a new source of confusion [38]. This stems from the fact that there are two different viewpoints when saying that an organism is a facultative/obligate mutualist/commensal/pathogen: the host perspective and the symbiont perspective. And in different fields the terms are applied from either perspective with opposing meaning. Most researchers would agree that *Buchnera aphidicola* is an obligate mutualist because it is unable to survive without its host and vice versa. However, the term facultative is almost always viewed from the host perspective by animal-microbe researchers, that is, the symbiont is not always needed by the host for survival (even if the bacterium itself is unable to survive outside the host). *Hamiltonella defensa* provides aphids with a selective advantage (protection from parasitoids) and is usually recognized as a facultative symbiont/mutualist because the host does not rely on it for survival [38]. Protistologists, in contrast, are often confused by this terminology and since *Hamiltonella* cannot survive outside its hosts, and, taking the symbiont perspective would therefore call such an organism an obligate symbiont. Accordingly, when describing any symbiont other than an obligate mutualist, proceed with caution! It may only be clear when you specify your viewpoint.

environment, which will have a large well-functioning genome, resetting the process [15,16,17••]. This pathway leads to the superficial appearance of an ancient endosymbiosis, but each individual endosymbiont was actually acquired relatively recently (Figure 1b). On the other hand, the host may establish protein import and sustain the symbiont with genes sheltered from extreme change in its own nuclear genome. These genes can be host-derived, but could also be acquired from many different donors (horizontal gene transfer; HGT), or from the endosymbiont (endosymbiotic gene transfer; EGT).
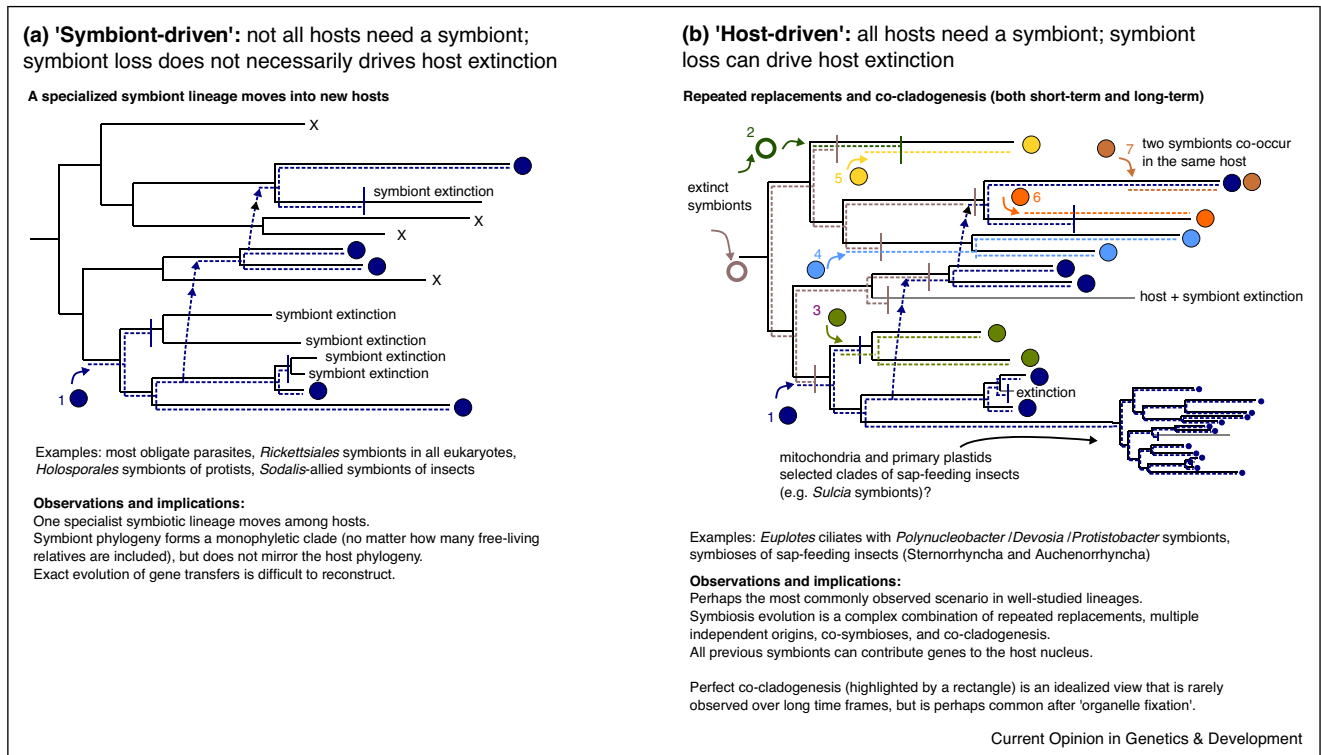
This pathway can lead to the fixation of organelles and is possibly the only option for the symbiont lineage to 'survive' for over time scales of billions of years, as have plastids and mitochondria. We note that by lineage survival, we mean 'genome survival' here as opposed to organismal/cellular survival (i.e. a cell lineage surviving by binary fission, including mitochondrion-derived organelles with no genomes) or gene survival (i.e. EGTs retained even after the organelle genome and its cellular compartment are lost).

In reality, a combination of these two pathways is likely required to explain fully fixed organelles. Indeed, developing a protein import machinery to sustain the recurring symbionts and fine-tune symbiont maintenance probably requires a long period of repeated endosymbiont replacement. Models for organelle origins that place an emphasis on this phase of transient symbiosis can be generalized as '**targeting-early**' models (e.g. the 'shopping bag' or 'targeting-ratchet' models [18,19]), as opposed to models where the symbiont is fixed before the origin of targeting, and are gaining support for plastids in particular. But the diverse origins of proteins targeted to other symbionts suggests this order of events applies to any host-symbiont system going down the rabbit hole [15,20••,21,22].

Significantly, any symbiont that falls in the rabbit hole almost always loses. It can slow its fall by outsmarting genome reduction (e.g. by HGT or strong selection on essential genetic machinery genes), but it will inevitably either go extinct or (very rarely) become an organelle. From the symbiont perspective, becoming an organelle is better than extinction, so it was 'lucky' [11]. But is it a win? Not necessarily. The ratchet does not stop at this point, only slows. Both plastids and mitochondria can still lose all their genes, or go extinct altogether [23,24•]. If the host fails to replace a poorly functioning symbiont or establish an organelle, it goes extinct together with the symbiont, but these cases will be invisible today.

The similarities in genetic integration that are emerging in plastids and bacterial endosymbionts have implications for the origin of eukaryotes as well. The mitochondrion is at the centre of a debate about whether its origin was a trigger for the origin of eukaryotic complexity in general, or if it was taken up by an already complex eukaryote-like cell [25,26•,27,28]. The recent identification of the Asgard superphylum [26•,29] has revised our view of the host. But the finding that mitochondrial-targeted proteins are not phylogenetically associated with the organelle and are instead derived from the host, various bacteria, archaea, or viruses [21,22,30], is also consistent with targeting-early models for genetic integration. At the same time, several distantly related protist lineages have been found to harbour gene-rich mitochondrial genomes [31•,32,33], showing that the ancestral

Figure 1

(a) 'Symbiont-driven': not all hosts need a symbiont; symbiont loss does not necessarily drives host extinction

A specialized symbiont lineage moves into new hosts

symbiont extinction
symbiont extinction
symbiont extinction
symbiont extinction
symbiont extinction

Examples: most obligate parasites, *Rickettsiales* symbionts in all eukaryotes, *Holosporales* symbionts of protists, *Sodalis*-allied symbionts of insects

Observations and implications:
One specialist symbiotic lineage moves among hosts.
Symbiont phylogeny forms a monophyletic clade (no matter how many free-living relatives are included), but does not mirror the host phylogeny.
Exact evolution of gene transfers is difficult to reconstruct.

(b) 'Host-driven': all hosts need a symbiont; symbiont loss can drive host extinction

Repeated replacements and co-cladogenesis (both short-term and long-term)

extinct symbionts

two symbionts co-occur in the same host

host + symbiont extinction

extinction

mitochondria and primary plastids selected clades of sap-feeding insects (e.g. *Sulcia* symbionts)?

Examples: *Euplotes* ciliates with *Polynucleobacter* /*Devosia* /*Protistobacter* symbionts, symbioses of sap-feeding insects (Sternorrhyncha and Auchenorrhyncha)

Observations and implications:
Perhaps the most commonly observed scenario in well-studied lineages.
Symbiosis evolution is a complex combination of repeated replacements, multiple independent origins, co-symbioses, and co-cladogenesis.
All previous symbionts can contribute genes to the host nucleus.

Perfect co-cladogenesis (highlighted by a rectangle) is an idealized view that is rarely observed over long time frames, but is perhaps common after 'organelle fixation'.

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Implications of control on evolutionary pathways in eukaryote-bacteria symbioses. We deliberately generalize and simplify here, to recognize two broad categories of symbioses based on the context of which partner is in control. **(a)** In 'symbiont-driven' associations, related hosts do not all require a symbiont for survival (and potentially derive no benefit from the symbiont), but the symbiont benefits from its host association. Endosymbionts can move between host lineages over evolutionary time, and the association can end without repercussions for the host. **(b)** In 'host-driven' endosymbioses all hosts need an endosymbiont for survival and reproduction. The inset shows one version of this where a stable association forms and the host and endosymbiont co-speciate. This stable association is often associated with host-dependency or co-dependency, but might actually be relatively rare over long time periods. Instead, as shown in the main tree, the host might take up and replace symbionts repeatedly over time, resulting in a pattern where the symbiosis is ancient, but all extant associations are less ancient. Both processes may contribute to the overall pattern of associations over long evolutionary time scales. We argue that these scenarios should be considered when discussing rates of host-symbiont extinction, horizontal/endosymbiotic gene transfer, symbiont losses, multiple independent origins, and replacements. In our view, the complexity of the Figure 1b represents the most likely trajectory for clades fully composed of obligate mutualists. This scenario can also lead to an early stage of organellogenesis.

mitochondrial genome was still relatively gene-rich, and that reduction took place many times in parallel, as has been observed in plastids [34]. Mitochondrial origins may therefore not require 'special' explanation, although its extreme age does leave more unanswered questions for both biological and methodical reasons [21,22].

Age and the symbiont-organelle transition

We have emphasized challenges imposed by the age of present-day mitochondria and plastids, but the fact they each originated once [35•,36] is equally problematic as it excludes most comparative analyses. Luckily, there are a variety of younger, analogous symbioses that originated many times independently in various hosts, and it is informative to compare these with more ancient events (Figure 2). For decades, however, the best-studied symbiotic systems of arthropods, protists, marine animals,

and other eukaryotes were viewed as lacking the genetic integration that was often argued to be the distinguishing feature of classic cellular organelles [37–39]. There was little evidence for host genes (either eukaryotic or HGTs) interacting with endosymbionts in any obvious or meaningful way, and no evidence of protein-targeting. Recent developments have changed this view entirely, suggesting that most, if not all, features previously used to define organelles occur in much younger systems [15,40,41,42••,43••,44,45].

Several bacterial symbionts of insects and protist that are tightly integrated with their host at the cellular and metabolic levels are now seen to be '*crossing the River Styx to the organelle world*' through the specific import of proteins from the host [41,43••,44,46]. The advent of protein import gives the host greater control over the

Figure 2
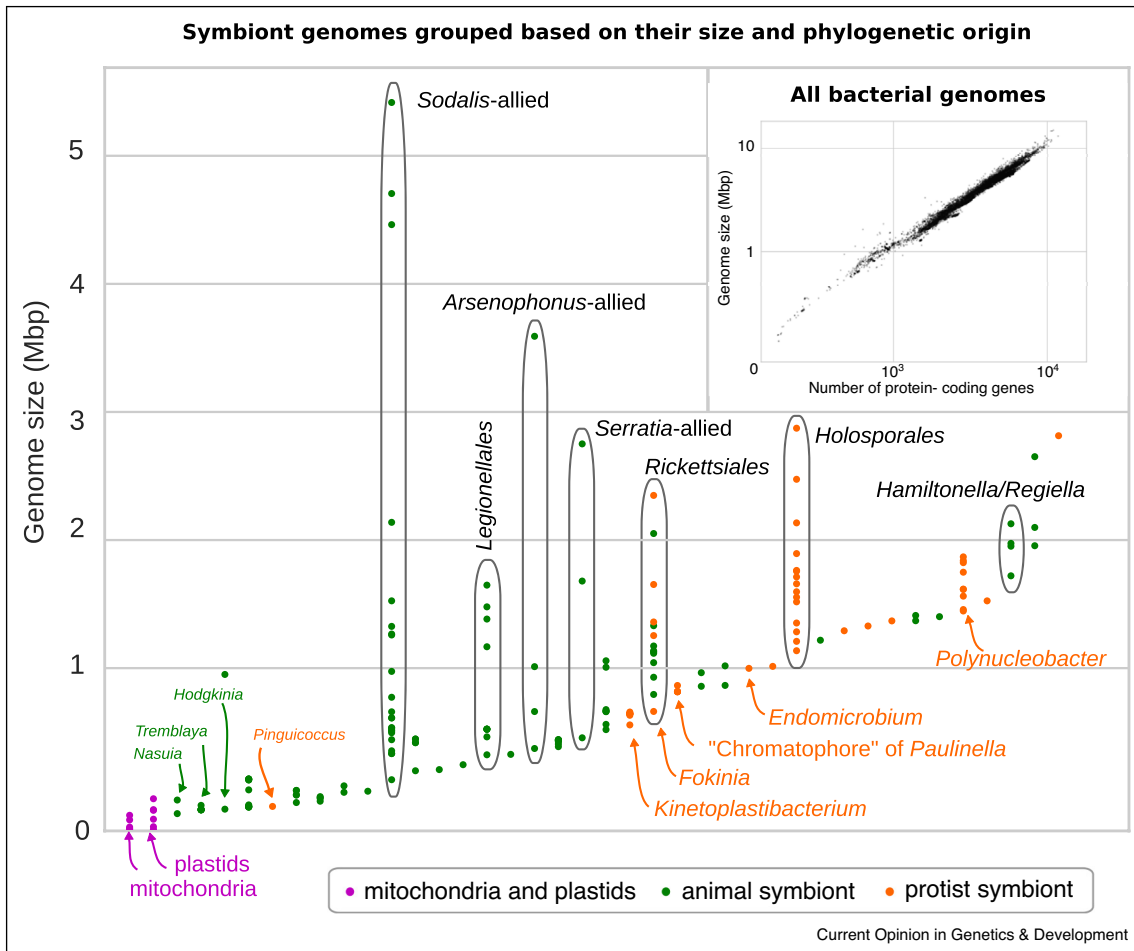
Extreme reduction in animal and protist endosymbionts: biology or bias? Bacterial genome size (Y-axis) typically correlates strongly with the number of protein-coding genes (X-axis of the inset). This relationship is shown broadly in the inset, and the correlation largely persists even in most reduced genomes (main panel, genomes simply sorted by size), all of which are from organelles and endosymbionts of animals and protists (colour-coded based on source, and circled based on phylogenetic origin when known to be from a strictly host-associated lineage specialized for symbiosis such as *Sodalis*-allied symbionts in insects). The most extreme cases of reduction in endosymbiotic bacteria rival gene-rich organelles (such as mitochondria of jakobids or plastids of red algae). However, these most reduced endosymbionts with one exception all come from animal-associated bacteria. This raises the possibility that insect endosymbionts are especially prone to this level of reduction due to some aspect of their biology, or perhaps more likely that similar extremes also exist in symbionts from single-celled eukaryotes, but they have not been found due to sampling bias for insect endosymbionts.

endosymbiont, but also creates an evolutionary ratchet where import of exogenous or host proteins render endosymbiont homologs obsolete, allowing the loss of otherwise essential functions such as DNA and RNA polymerases and translation-related genes (Figure 3). The endosymbiont can become part of its host cell— an organelle. Exactly how these proteins are targeted remains an important question: the import system may be derived from that of existing organelles or use already established endomembrane trafficking pathways (especially in cases where the outer membranes of some of these symbionts are entirely host-derived [15]). Outer membrane vesicles are also critical elements in many extracellular host-microbe interactions, such as the squid-*Vibrio* or human-gut microbiota [47,48], but their role in organellogenesis remains enigmatic [49].

Neglected protist models for symbioses

Simple logic would suggest that we should most often find organelle-like endosymbionts in unicellular eukaryotes. These eukaryotes are commonly bacterivorous and both transmission and domestication of endosymbionts should be more straightforward since they lack a protected germline. Indeed, this is exactly what we envision the archaeplastidal ancestor that acquired plastids to be like

Figure 3
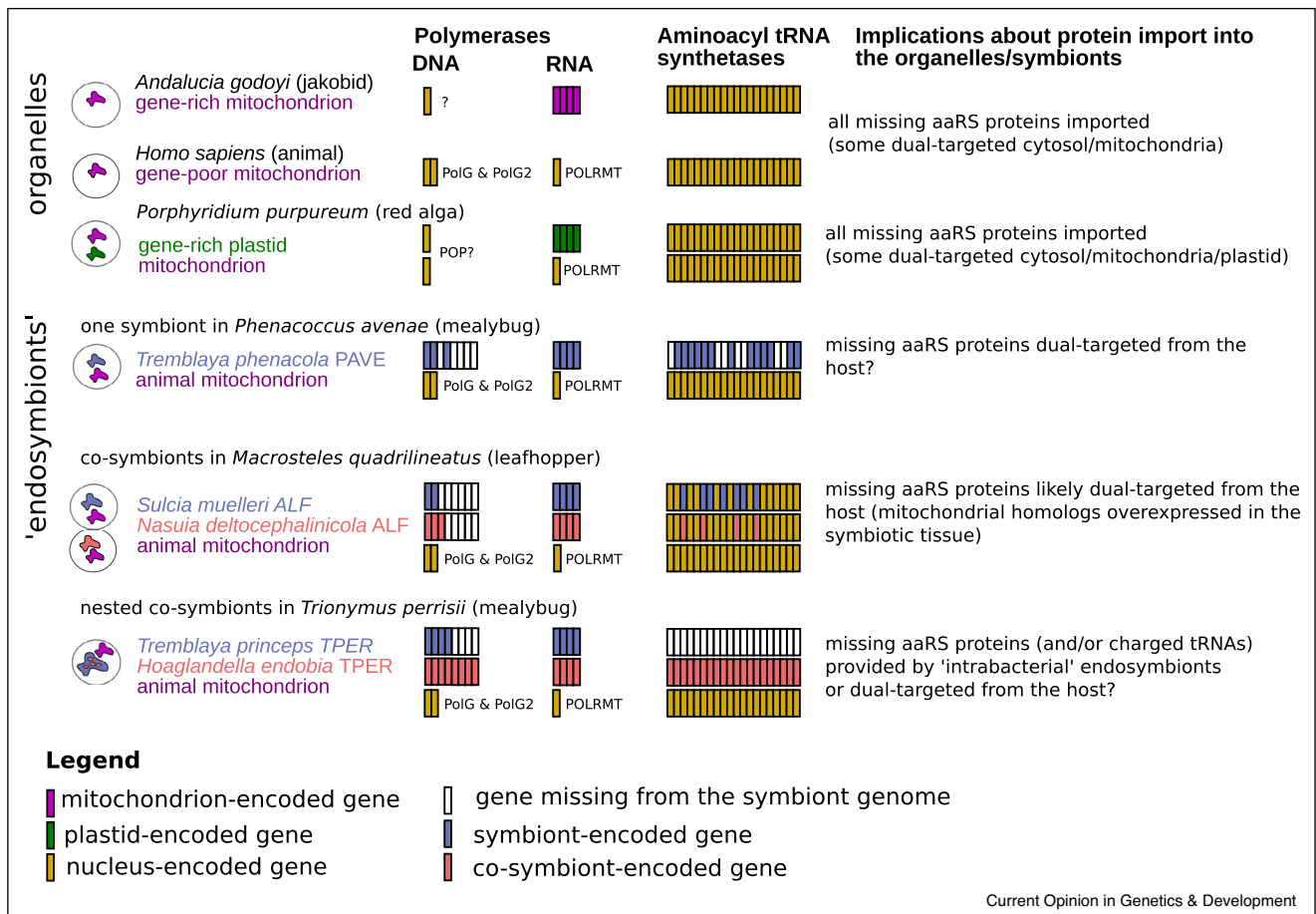
Protein import in extremely reduced endosymbionts. The genomes of several organelles (top) and highly reduced endosymbionts (bottom) are missing genes known to be essential for core cellular processes like DNA replication or translation (represented here by DNA polymerases and aminoacyl tRNA synthetases). Many endosymbiont and organelle researchers would agree that the point when an endosymbiont becomes organelle-like is when there is a well-established system for protein import from the host. This reasoning stems from what was thought to be a distinguishing feature of mitochondria and plastids—that most of their proteins are expressed in the host cytoplasm and specifically targeted to the organelle. There are now several examples of proteins imported into other endosymbionts as well, but none of them is from an essential cellular system such as translation or DNA replication. However, genes related to these processes are clearly missing from their genomes, suggesting they must be somehow imported. A comprehensive analysis of metabolite and protein exchange at the host-symbiont interfaces in complex systems will be methodologically challenging, but could answer perhaps the most important question of the field: which host proteins are imported into endosymbionts and how? Colored boxes represent the following bacterial genes (unless stated otherwise): *dnaEQNX*, *holABCD*, *rpoABCD*, *argS*, *cysS*, *glnS*, *gltX*, *ileS*, *leuS*, *valS*, *alaS*, *asnS*, *aspS*, *tyrS*, *trpS*, *thrS*, *serS*, *proS*, *pheST*, *metG*, *lysS*, *hisS*, *glySQ*.

[50]. Although more debated, the ancestor that took up mitochondria was plausibly similar as well.

So why don't we find numerous 'novel organelles' in protists, and why are the protist endosymbionts we do know less reduced than those from insect systems (Figure 3)? Perhaps the simple answer is lack of sampling, especially in comparison to insects. As a model system, nutritional symbionts of insects have become disproportionately sampled in the race to characterize maximal reduction. Despite this sampling bias, however, possibly the most striking cases of a new 'organelle' is from a protist. The symbiotic cyanobacterium (called a chromatophore or cyanelle) in the Rhizarian *Paulinella chromatophora* is functionally analogous to plastids, but originated only 90–140 million years ago. Despite its young age and modest genome reduction (1 021 616 bp), it seems to be on the path to becoming genetically integrated and already depends on massive protein import from the host cytoplasm [43[••],51]. Strikingly, out of 229 bacterial genes in *Paulinella*, only about 25% putatively arose through EGT from the symbiont genome [20[••]].

As more endosymbionts from protists are characterized, the observed levels of reduction are also closing in on the

most extreme cases in insects (Figures 2 and 3), but whether they will eclipse them or if there is something about the animal systems that favours extreme reduction is as yet unclear. Indeed, broader sampling should make a major impact on our narrow view of the functions that underpin endosymbiosis more generally. Current model systems are heavily dominated by nutritional endosymbionts of animals with restricted diets, and similar needs do drive protist hosts as well: betaproteobacteria provide amino acids and vitamins to trypanosomatids (living in insects feeding on plants and blood) [52,53], and *Endomicrobium* assists termite protists digesting cellulose [54]. But the functional basis for most protist symbioses are completely unknown, and unlikely to be nutritional. Protist metabolism is not as restricted in scope as that of animals, and many compounds provided by animal symbionts can be synthesized by most protist hosts. Furthermore, the bacterivorous lifestyle of many protists provides a diet that could hardly be more different from nutritionally unbalanced diets of the best-studied animal hosts of endosymbionts (which feed on plant sap, blood, or wood). Other aspects of protist biology that differ from animals might also affect the likelihood of different pathways open to their endosymbionts. For example, their constant feeding on bacteria increases the chance of replacing degenerating endosymbiont, whereas their fast generation times (hours or days versus weeks or years in insects) and lack of germ line both affect the relative importance of different forces acting at the level of endosymbiont populations.

Where we are, and looking forward

Observation and theory based on data from organelles and bacterial endosymbionts of animals are finally beginning to merge on a more unified picture of endosymbiosis as a process, with some surprising results. Probably the most important are the fundamental changes in how we interpret the process at a mechanistic level (e.g. the order of events and importance of transient stages), and the implications this has for how the partners evolve and adapt (e.g. the dominance of conflict over cooperation and how the relationship is heavily context-dependent). The next few years should see these ideas tested rigorously and, if they survive this scrutiny, the evolutionary underpinnings of endosymbiosis and its impacts on the tree of life will undergo their greatest revision since the acceptance of endosymbiotic organelle origins. Less clear is how our views of the functional basis for these relationships might change. Opening the Pandora's box of protist diversity may similarly challenge our ideas about symbiotic function, but this has the potential to be extremely difficult to resolve since the range of possible functions and how to infer them from the kinds of data we are good at generating (e.g. sequencing) are difficult to predict. One symbiont-derived function that could be universally important is defence, but the range

of other possible functions in the context of a single celled host is potentially only limited by our imaginations.

Conflict of interest statement

Nothing declared.

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- •• of outstanding interest

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