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Minireview

Interspecific evolution: microbial symbiosis, endosymbiosis and gene transfer

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Summary

Microbial symbioses are interesting in their own right and also serve as exemplary models to help biologists to understand two important symbioses in the evolutionary past of eukaryotic cells: the origins of chloroplasts and mitochondria. Most, if not all, microbial symbioses have a chemical basis: compounds produced by one partner are useful for the other. But symbioses can also entail the transfer of genes from one partner to the other, which in some cases cements two cells into a bipartite, co-evolving unit. Here, we discuss some microbial symbioses in which progress is being made in uncovering the nature of symbiotic interactions: anaerobic methane-oxidizing consortia, marine worms that possess endosymbionts instead of a digestive tract, amino acid-producing endosymbionts of aphids, prokaryotic endosymbionts living within a prokaryotic host within mealybugs, endosymbionts of an insect vector of human disease and a photosynthetic sea slug that steals chloroplasts from algae. In the case of chloroplasts and mitochondria, examples of recent and ancient gene transfer to the chromosomes of their host cell illustrate the process of genetic merger in the wake of organelle origins.

Introduction

Biologists often use the word 'symbiosis' in the sense of mutualism, that is living together for the benefit of both partners. But the original meaning, as coined by Anton DeBary and Simon Schwendener about 150 years ago from their work on lichens (associations of fungi with photosynthesizers), is simply 'living together'. Fifty years ago, Buchner's (1953) seminal book catalogued countless fascinating examples of microbes living within animal cells. Today, symbioses are as interesting as ever, and many are now becoming better understood in terms of their physiological basis, that is who is getting what from whom? The purpose of this paper is to point out a few such examples in the hope that readers find them interesting, as a motivation for further reading.

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Anaerobic methane-oxidizing consortia – a glance at ancient ecosystems?

Anaerobic methane-oxidizing consortia have made quite a bit of news lately. As pointed out recently in these pages (Valentine and Reeburgh, 2000), anaerobic methane oxidation is a microbial process of global importance in marine sediments, and the nature of the microbes that perform this process has been a long-standing mystery. Probing the environment with molecular tools, Ed DeLong and colleagues found a new group of methanogens, relatives of the Methanosacinales, in marine sediments associated with eubacterial lipids, which carried the distinctive isotope imprint of methane-derived carbon, suggesting that a complex microbial community rather than a single microbe might be catalysing anaerobic methane oxidation (Hinrichs *et al*., 1999). Antje Boetius and colleagues (Boetius *et al*., 2000) found that symbiotic consortia of archaea and sulphate-reducing bacteria are probably the biological agents of anaerobic methane oxidation. The consortia are found in methane hydrate-rich marine sediments and consist of tight packets of about 100 archaebacterial cells surrounded by about 200 cells of sulphate-reducing eubacteria (Fig. 1), as revealed by fluorescent *in situ* hybridization (FISH). The archaebacterial partners are methanogen relatives on the basis of their rRNA sequences, whereas the eubacterial partners seem to be closely related to the *Desulfosarcina* group of sulphate reducers. Although the biochemical details are by no means clear, the suspicion is that the sulphate reducers are such avid consumers of H_2 that the archaebacterial partners might be performing a variant of methanogenesis in the methane-consuming rather than methane-producing direction (Boetius *et al*., 2000), but this has yet to be shown.

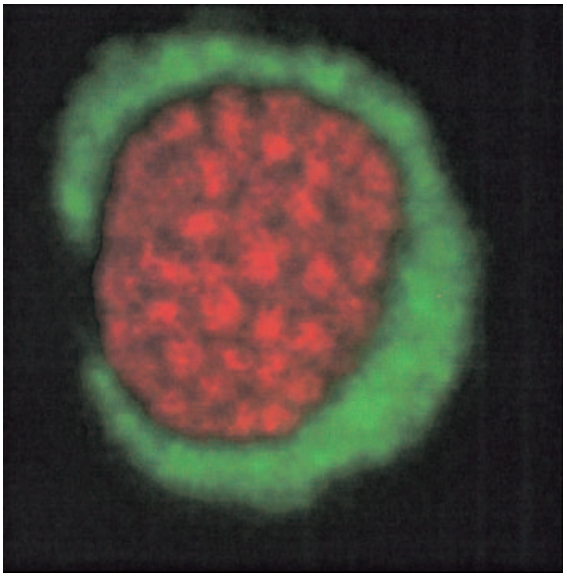

Fig. 1. Anaerobic methane-oxidizing consortia consisting of methanogens (red) and sulphate reducers (green) as revealed by FISH imaging (see Boetius *et al*., 2000). Figure kindly provided by Antje Boetius.

More recently, Michaelis *et al*. (2002) reported anaerobic methane-oxidizing consortia from the anaerobic waters of the Black Sea, which build up massive magnesium carbonate reefs. In contrast to the previous example, here, the archaebacterial partners seem to surround the sulphate reducers, as revealed by FISH micrographs. The striking carbonate reefs, up to 1 m high, consist largely of methane-derived carbonate, as indicated by the ultralight carbon isotope composition of the carbonate. Furthermore, pieces of reef material incubated *in vitro* with [^{14}C]-methane yielded incorporation of ^{14}C into acid-labile carbon (carbonate) (Michaelis *et al*., 2002). Again, the lipids of the sulphate reducer carried the characteristic trace of methane-derived carbon.

From the evolutionary standpoint, a particularly exciting aspect of the anaerobic methane-oxidizing consortia is that they may be providing a glimpse into the microbiology of some of the earth's most ancient ecosystems. The isotopic trace of sulphate reduction has recently been found in 3.4-billion-year-old sedimentary rocks (Shen *et al*., 2001). The isotopic record of ultralight carbon, generally believed to be an indicator of methanogenesis, goes back at least 2.7 billion years (Hayes, 1994). Anaerobic methane oxidation may thus have been a globally widespread process of enormous ecological importance during the anaerobic phases of earth's history. As molecular oxygen did not appear in the earth's atmosphere until about 2 billion years ago (Nisbet and Sleep, 2001), and as the earth's deep ocean water may have been anoxic up until about 1 billion years ago (Anbar and Knoll, 2002), anaerobic methane oxidation – possibly involving intimate symbioses of archaebacterial and eubacterial partners – may have been globally widespread in marine sediment communities for the majority of earth's history.

Worms with endosymbionts instead of a digestive tract

Although anaerobic marine sediments are interesting, oxygen-poor environments closer to the earth's surface also harbour fascinating symbioses. For example, the marine oligochaete worm *Olavius algarvensis* is about 3 cm long and lives in the shallow sands of Mediterranean shores. It is unusual in that it possesses no mouth or digestive tract at all, but rather lives from the reduced carbon produced by the action of a consortium of two different endosymbiotic bacteria that live in the epithelium just below the worm's outer surface (Fig. 2). Using the FISH technique, Dubilier *et al*. (2001) showed that the consortium consists of a smaller δ-proteobacterium related to the sulphate reducer *Desulfosarcina variabilis* and a larger γ-proteobacterium related to *Allochromatium vinosum*. Similar γ-proteobacterial symbionts, chemoautotrophs, have been known for some time in other marine worms (Dubilier *et al*., 1995) and, in some cases, were shown to be sulphide oxidizers. The physiological role of such γ-proteobacterial symbionts is to produce reduced carbon compounds from which their worm hosts gain their ATP through heterotrophy. In this sense, the role of the γ-proteobacterial symbionts seems to be chemosynthate production for the worm, in analogy to photosynthate produced by plastids for plants. But, in the case of *Olavius*, what are the δ-proteobacteria doing inside the worm? Using an elegant technique, Dubilier *et al*. (1995) showed that, under microaerobic conditions in the laboratory, $^{35}SO_4^{2-}$ is reduced, probably by the δ-proteobacterium. The reduced radioactive sulphide can be detected by a thin silver needle that is inserted into the worms, upon which the sulphide precipitates, allowing detection by autoradiography. This technique reveals that sulphate is being reduced to sulphide inside the worm.

In agreement with its likely role in sulphate reduction, the δ-proteobacterium possesses a gene for dissimilatory sulphite reductase (DSR), which reduces sulphite to sulphide in sulphate reducers and is a good indicator of sulphide production. The sulphide, which would be toxic to the worms at high concentrations, does not accumulate within the animal, rather it is apparently oxidized back to sulphate by the γ-proteobacterium, suggesting the presence of a syntrophic sulphur utilization cycle between these two prokaryotes inside the worm. In line with that view, the estimated flux of environmental sulphide into the worms based on pore water sulphide concentrations measured in their habitats was much lower than the 'internal'

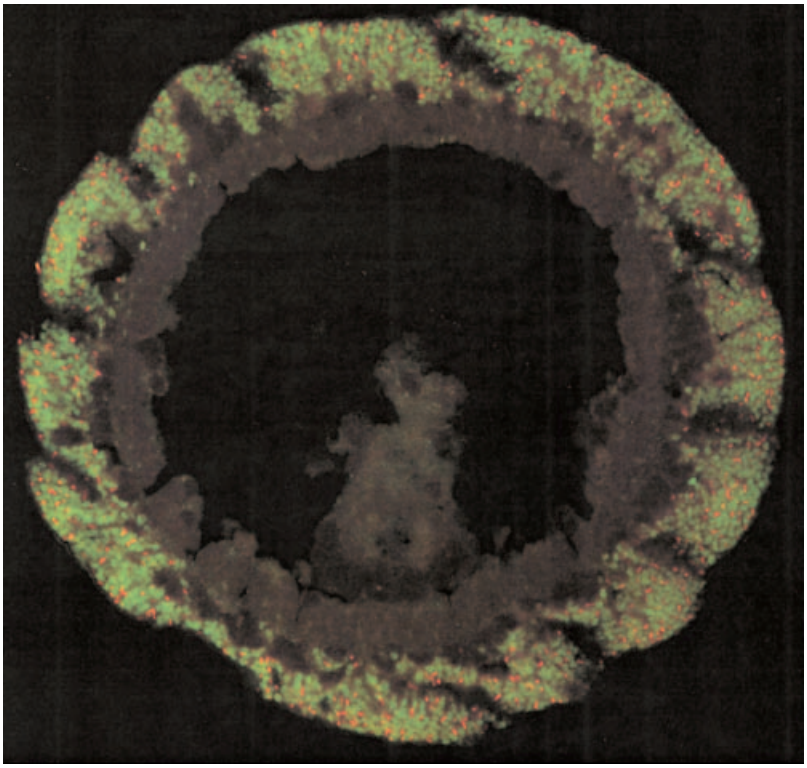

Fig. 2. Microbial consortia in the epithelium of the gutless oligochaete *Olavius algarvensis* as revealed by FISH imaging in a cross-section (see Dubilier *et al*., 2001). The γ-protobacterial endosymbionts are labelled green, the δ-proteobacterial endosymbionts are labelled red. Figure kindly provided by Nicole Dubilier.

sulphide production from the endosymbiotic sulphate reducers. On the bottom line, it appears as though the γ-proteobacterial endosymbionts in the gutless worm *Olavius* provide their small metazoan host with carbohydrate produced through chemoautotrophy with electrons stemming from sulphide, which the δ-proteobacterial endosymbionts provide using electrons stemming from H_2 or organics (Dubilier *et al*.. 2001).

Closer to home – obligate eubacterial endosymbionts of aphids

Rose gardeners (and many people who park their cars under trees in summer) know that aphids make a living from sucking the sugar-rich phloem sap of higher plants. But phloem is generally poor in amino acids and, to compensate for that, many aphid species harbour endosymbiotic eubacteria. In the case of the pea aphid, symbiosis is obligate: the aphids cannot live without their endosymbionts nor can the endosymbionts be cultured outside their insect host (Zientz *et al*., 2001). The genome of the pea aphid symbiont, the γ-proteobacterium *Buchnera* sp., was published not too long ago and revealed some dramatic insights into the biology of this symbiosis (Shigenobu *et al*., 2000). The *Buchnera* genome is only about one-seventh the size of the *Escherichia coli* K-12 genome and is present in more than 100 copies per cell. Earlier work had shown that the biological association between the bacterial symbionts and the aphid lies in amino acid production (Lai *et al*., 1994). The symbionts overproduce some amino acids, which they export to their aphid hosts. But the *Buchnera* genome sequence revealed unexpected surprises.

The *Buchnera* genome encodes 54 genes involved in amino acid synthesis – but only for the synthesis of essential amino acids, that is those that the aphids are unable to synthesize themselves (Shigenobu *et al*., 2000). Genes for the synthesis of non-essential amino acids are missing altogether in the *Buchnera* genome, which can mean but one thing. The endosymbiotic bacteria must be obtaining their non-essential amino acids from their insect host. The aphid's reward for providing a life support system for its bacterium seems to be founded in reciprocity, namely the uptake of essential amino acids that are produced by the symbiont. As some non-essential amino acids are precursors for the synthesis of essential amino acids, the biosynthetic pathways of the host and symbiont are inextricably intertwined (Shigenobu *et al*., 2000).

According to the genome data, *Buchnera* seems to respire oxygen provided through the aphid's trachea system. *Buchnera* possesses all genes necessary for glycolysis, pentose phosphate pathway and aerobic respiration, but almost all the genes for the TCA cycle are missing. In addition, *Buchnera* has retained only few genes for DNA repair (Shigenobu *et al*., 2000), which might help to explain why it has accumulated so many mutations rela-

tive to *E. coli* in phylogenetic comparisons (Itoh *et al*., 2002). *Buchnera* depends on nutrients from the host and provides nutrients to the host, also by biochemically processing precursors that the host provides. Earlier evolutionary studies revealed that aphids and their endosymbionts have undergone symbiotic co-evolution for perhaps as long as 200 million years (Moran and Baumann, 1994). Indeed, the endosymbionts are passed along from one generation to the next through the egg cells. In many ways, this endosymbiosis is strikingly similar to the physiological integration of mitochondria or chloroplasts in eukaryotic cells. Is *Buchnera* a new amino acid-producing organelle in the making? Maybe, but then again, perhaps more probably not. In contrast to chloroplasts and mitochondria, *Buchnera* exists only in one type of highly specialized cells (bacteriocytes) within a highly specialized organ tissue (the bacteriome) of the aphid host, whereas chloroplasts and mitochondria are integrated into the physiology of every host cell. Nonetheless, the level of biochemical integration achieved by aphids and the bacterial symbionts is a striking example of physiological integration of two different organisms into a now indivisible unit.

Prokaryotes within prokaryotes within eukaryotes: mealybug symbionts

Many fanciers of house plants have made the acquaintance of mealybugs: small white, cotton ball-like insects that, like aphids, live from sucking sugar-rich plant sap. Mealybugs possess proteobacterial endosymbionts in bacteriocytes within a bacteriome, much as in the case of aphids mentioned above. But recent work by von Dohlen *et al*. (2001) confirmed with *in situ* hybridization that, in contrast to the case of aphids discussed above, the mealybug endosymbionts were β-proteobacteria and – much more surprisingly – revealed that γ-proteobacterial endosymbionts live inside the β-proteobacterial endosymbionts. That is, they found one prokaryote living as an endosymbiont within another (Fig. 3). This appears to be a hitherto unprecedented case of a prokaryotic endosymbiont within a prokaryotic host, a remarkable example of endosymbiosis. Transmission electron microscopy revealed that the β-proteobacterial host cell cytoplasm is separated from the γ-proteobacterial symbiont cytoplasm by two membranes, whereby β-proteobacterial cytosol is separated from the insect host cytosol by three membranes (presumably two belonging to the bacterium and one stemming from the insect host). The symbiosis is stable throughout the life cycle, and the double-decker symbionts are apparently passed along to mealybug offspring (von Dohlen *et al*., 2001), but the biochemical–physiological basis of this intriguing symbiosis is not yet understood.

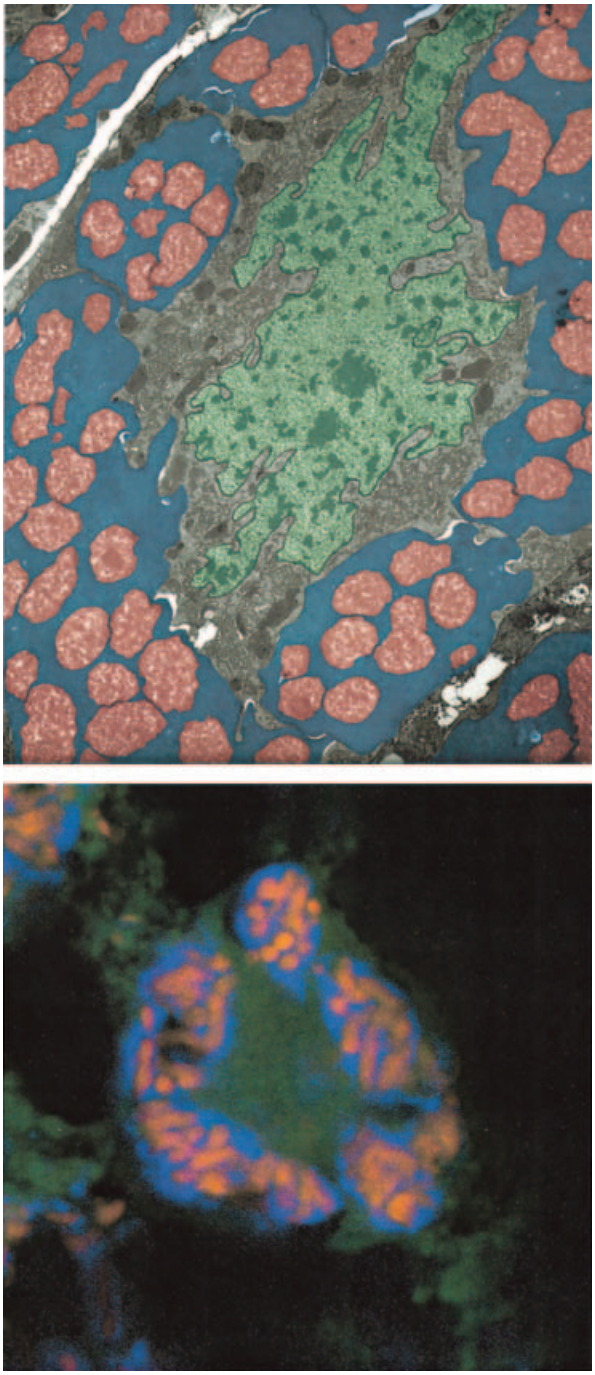

Fig. 3. Cells within cells within cells (see von Dohlen *et al*., 2001).
Top. False-colour electron micrograph of a mealybug (*Planococcus citri*) cell showing the nucleus (shaded green) surrounded by large β-proteobacterial endosymbionts (shaded blue), which contain smaller γ-proteobacterial endosymbionts (shaded red).
Bottom. False-colour FISH image of the mealybug endosymbionts, showing γ-proteobacterial endosymbionts (red) within their β-proteobacterial host (blue), all within the confines of a mealybug cell (background fluorescence, including nucleus, shaded green). Both figures kindly provided by Carol von Dohlen.

Curiously, eubacteria are immensely common as endosymbionts among eukaryotes, whereas archaebacteria are not. Archaebacteria occur only very rarely as endosymbionts in eukaryotes, although 'rarely' might be the wrong term here, because archaebacterial symbionts are actually quite widespread among eukaryotes but, until now, they have been represented only by the methanogens and only as endosymbionts in anaerobic eukaryotes that contain hydrogenosomes, for example among the ciliates (Embley *et al*., 1995; Fenchel and Finlay, 1995), where the methanogens live from the H_2 produced in hydrogenosomes – anaerobic forms of mitochondria. More examples of archaebacterial endosymbionts may come to light as new biological systems are explored.

The eubacterial symbionts of insects alone are turning out to be much more widespread in nature than previously assumed, and many of them have an impact upon humans that goes far beyond rose gardens and house plants (Zientz *et al*., 2001). For example, the dreaded tsetse fly also possesses eubacterial symbionts. Tsetse flies are the insect vectors that transmit African trypanosomes, the infectious agents of many tropical diseases such as African sleeping sickness, which affects millions of humans each year. The tsetse fly endosymbiont is *Wigglesworthia glossinidia*, a γ-proteobacterium that, like *Buchnera*, possesses a highly reduced genome. However, in contrast to *Buchnera*, the physiological basis of the interaction between *Wigglesworthia* and its insect host seems to be vitamin biosynthesis, rather than amino acid biosynthesis, as suggested by the genome sequence (Akman *et al*., 2002) and by the fact that animal blood, the tsetse fly's main diet, is very poor in vitamins. To make things more complicated, the trypanosomes that are transmitted by tsetse flies seem to have possessed their own surprising endosymbiont, as gene sequence comparisons now reveal evidence for the past presence of a plastid in the trypanosome lineage (Hannaert *et al*., 2003), which apparently has been secondarily lost through reduction, but only after it transferred some genes to its host (see below).

Elysia — the photosynthetic slug with stolen chloroplasts

Not only can plastids become secondarily lost, they can be secondarily acquired, because many groups of photosynthetic protists harbour plastids that they obtained not from cyanobacteria, but from eukaryotic algae instead, a process known as secondary endosymbiosis (Stoebe and Maier, 2002). However, some rather remarkable heterotrophic hosts are very choosy when it comes to obtaining plastids. Rather than incorporating the plastid into their cells to be passed on from generation to generation, they go out and get a fresh helping of plastids each year, as work by Rumpho *et al*. (2000) has shown in the case of the fascinating symbiotic association found between molluscan sea slugs from the genus *Elysia* and their algal chloroplasts. The sea slug *Elysia chlorotica* preferentially feeds upon the siphonaceous xanthophycean alga *Vaucheria litorea*, which possesses roughly centimetre-sized, multinucleate (syncitial) cells with cytoplasm that is full of bright green plastids. The sea slugs puncture the algal cell and suck out the plastids. But rather than digest the plastids for a straightforward meal, *Elysia* sends the plastids through a specialized, ramified digestive system located one cell layer beneath the epidermis. The plastids are maintained there in a functional form for several months (!), and the slug uses their photosynthesis, giving the animals their distinctive green colour (Fig. 4). Not whole algal cells, but only their plastids are maintained. The plastids (or the green slugs, depending on how one views it) produce O_2 in a light-dependent manner and fix CO_2. In fact, the standard culture procedure for the slugs, once they have obtained their plastids, is an aquarium supplied with only light and CO_2 (Rumpho *et al*., 2000).

The chloroplasts that *Elysia* borrows from the algae perform protein synthesis (Green *et al*., 2000). The big mystery is still how the plastids remain photosynthetic for months. So far, neither residual algal nuclei nor the existence of algal DNA has be detected in *E. chlorotica*. Given the high turnover of proteins normally found in photosynthetic reaction centres, and given that many components of the photosynthetic membrane are nuclear encoded

Fig. 4. The sea slug *Elysia chlorotica* associated with newly produced (non-pigmented) eggs (see Rumpho *et al*., 2000). The animal's green colour results from plastids that the slug captures from an alga and maintains for months in a photosynthetically active state. Figure kindly provided by Mary Rumpho.

(Allen, 2002), it seems that many essential proteins must somehow be imported to maintain the activity of the plastids. Several hypotheses are currently discussed concerning how the long-term photosynthetic activity of the symbiont is maintained in the special case of *E. chlorotica* and *V. litorea* (Rumpho *et al*., 2000), but the underlying mechanisms are still unknown. Regardless of how the plastids are maintained, this recurrent symbiosis is also obligate because, if juvenile slugs do not acquire their plastids, they do not develop into viable adults (West *et al*., 1984).

Gene transfers recent and ancient, perhaps most importantly from organelles

In the symbioses mentioned above, there has been ample opportunity during evolution for genes to have been exchanged between the symbiotic partners, so many researchers are now on the lookout for such laterally transferred genes. Indeed, Kondo *et al*. (2002) found that the insect endosymbiont *Wolbachia*, a γ-proteobacterium that inhabits numerous insect species, seems to have recently transferred an ≈11 kb fragment of bacterial DNA to the nuclear genome of the adzuki bean beetle *Callosobruchus*. However, in the case of chloroplasts and mitochondria, the endosymbiosis is much older than any of the insect–eubacterium symbioses known, so even greater opportunities for gene transfer have existed. Phytophagous insects must be much younger than the land plants upon which they depend, and land plants are only about 450 million years old. In contrast, plastids are at least 1.2 (Butterfield, 2000) and possibly 1.5 (Javaux *et al*., 2001) billion years old, and mitochondria must therefore be even older, having arisen perhaps some 2 billion years ago (Martin and Russell, 2003).

Lateral gene transfers from organelles to the nucleus, both recent and ancient, are very common indeed. For example, the *Arabidopsis* genome revealed what seemed to be an ≈270 kb piece of the mitochondrial genome integrated on chromosome 2 that was 99% identical to the genuine mitochondrial genome (Lin *et al*., 1999). Subsequent work (Stupar *et al*., 2001) revealed that the transfer actually encompassed the entire 367 kb mitochondrial genome, including an unusual duplication that had been missed in the *Arabidopsis* sequence assembly. This transfer probably occurred of the order of 2 million years ago (Henze and Martin, 2001). Yet, similarly recent large transfers of chloroplast DNA were quite rare in the *Arabidopsis* genome (The *Arabidopsis* Genome Initiative, 2000). However, it can be expected that other higher plant or algal genomes might possess more in the way of recently transferred chloroplast DNA. Supporting this view, a recent study revealed a 33 kb chunk of transferred chloroplast DNA on the long arm of rice chromosome 10 that is 99.7% identical to the chloroplast-localized molecule (Yuan *et al*., 2002). But transfer of smaller pieces of DNA are also an ongoing process, as broad-scale searches for transferred genes among higher plants have shown (Adams *et al*., 2002).

Transfers from organelles to the nucleus – both recent and ancient – are probably much, much more abundant than previously assumed. A recent genome-wide study revealed that about 18% of the nuclear-encoded proteins in *Arabidopsis* come from cyanobacteria, that is from the ancestral plastid genome (Martin *et al*., 2002). That is a surprisingly large contribution from the plastid. Yet the more burning question of how many genes in eukaryotes ultimately come from the ancestral mitochondrial genome is currently under heavy debate. Genome-wide surveys have revealed that more than half the genes in the yeast genome are more similar to eubacterial than to archaebacterial homologues (Rivera *et al*., 1998; Horiike *et al*., 2001), and similar findings have been reported for the mitochondrion-lacking protist *Giardia intestinalis* (Hartman and Fedorov, 2002) (for a different perspective on these findings, see Rotte and Martin, 2001).

These reports of 'too many' eubacterial genes in eukaryotes are vexing to evolutionary microbiologists, because the widely accepted Woese–Kandler–Wheelis (Woese *et al*., 1990) domain classification scheme would have us expect eukaryotes, in essence, to be archaebacteria. Do all these eubacterial genes in yeast and *Giardia* come from mitochondria or have there been other donors as well?

Some authors lean towards the view that all the eubacterial genes in eukaryotes that do not branch specifically with α-proteobacterial homologues are acquisitions from other sources, for example as Baughn and Malamy (2002) argued for some citric acid cycle enzymes. The most popular generalized variant of this kind of rampant lateral acquisition model implicates as the donor a hypothetical 'mystery endosymbiont' that preceded the mitochondrion (Horiike *et al*., 2001; Hedges *et al*., 2001; Hartman and Fedorov, 2002). One of many problems with the view of a premitochondrial mystery symbiont is that the 'too many' eubacterial genes in eukaryotes do not branch with homologues from any particular eubacterial lineage, rather they branch with homologues from all kinds of lineages. That is, the genes that branch with α-proteobacterial homologues are viewed as coming from the mitochondrion, the others are viewed as coming from the earlier 'mystery donor', even though no contemporary eubacterium is known to possess such a combination of genes.

Another variant to account for the 'too many' eubacterial genes in eukaryotes is the view that all the eubacterial genes in eukaryotes that do not branch specifically with α-proteobacterial homologues are acquisitions from separate donors. At the extreme, even nuclear genes that do

branch specifically with α-proteobacterial homologues could be explained as lateral acquisitions from phagocytosed α-proteobacteria bacteria, rather than from the mitochondrion (Doolittle, 1998). One problem with this view is that different eukaryotic lineages would be expected to have acquired very different sets of eubacterial genes, but studies of several typically eubacterial-but-non-α-proteobacterial eukaryotic genes such as [Fe]-hydrogenase and pyruvate:ferredoxin oxidoreductase point to a single acquisition in the common ancestor of all eukaryotes, rather than multiple acquisitions (Embley *et al*., 2003).

Less radical, but also less popular, is the view that the 'too many' eubacterial genes in eukaryotes simply come from the mitochondrial endosymbiont. Under that view, the fact that poorly conserved genes do not perform well in phylogenetic analyses (Martin *et al*., 2002) and the reality of lateral gene transfers between free-living eubacteria (Gogarten *et al*., 2002) would just as easily account for the myriad of *apparent* eubacterial donors to eukaryotic genomes, but without invoking a new endosymbiosis and transfer event for each non-α-proteobacterial branch (Martin, 1999).

Another factor in this issue concerns the assumptions one makes about the eubacterium that was the ancestor of mitochondria. The α-proteobacteria are an extremely diverse group. Some authors strongly favour the view that the ancestor of mitochondria was a highly reduced obligate aerobe such as *Rickettsia* (Andersson and Kurland, 1999), in which case all anaerobic functions in mitochondria, for example, would have to be lateral transfers. Others favour the view that the ancestor of mitochondria was a facultative anaerobe, perhaps such as *Rhodobacter*, which would fit better as a donor for the biochemistry of anaerobic mitochondria and hydrogenosomes, their H_2-producing relatives (Tielens *et al*., 2002).

Sediments and organelles, now and then

Biochemical insights into modern symbioses hold the key to a tangible grasp of ancient symbioses as the origin of eukaryotic organelles. But, regardless of whatever kind of eubacterium the ancestor of mitochondria was, it lived some 2 billion years ago, it is not alive today, only some descendants are. Because of lateral gene transfer, it is doubtful that any contemporary eubacterium possesses exactly the same set of genes as that prokaryote did when it became an endosymbiont. In fact, the whole chemistry of earth was different 2 billion years ago (Nisbet and Sleep, 2001). At that time, oxygen was just beginning to appear in the atmosphere, and newer findings indicate that deep ocean water was still anoxic and was furthermore laden with sulphide produced by sulphate reducers (Anbar and Knoll, 2002). Conceivably, much of the earth's marine microbial community then looked much like the Black Sea does today. Given that both the ancestor of mitochondria and its host lived in a world where oxygen was rare, it seems reasonable to assume that both of them were fully suited to anaerobic environments. In today's eukaryotes, relicts of that anaerobic past have persisted in the biochemistry of anaerobic mitochondria and hydrogenosomes (Tielens *et al*., 2002; Embley *et al*., 2003) and in mitochondrial sulphide oxidation (Doeller *et al*., 2001; Yong and Searcy, 2001). Modern anaerobic marine environments such as the Black Sea (Michaelis *et al*., 2002) or the Santa Barbara basin (Bernhard *et al*., 2000) harbour a myriad of microbial symbioses awaiting further study.

Geologists have long been guided by Hutton's principle of uniformitarianism – that processes observable today should have operated long ago as well ('the present is the key to the past'). Biologists, on the other hand, have been guided by Darwin's principle of descent with modification – that variation among offspring and natural selection can fully account for the diversity of life. Between these pillars, the concept of endosymbiosis in evolution (Mereschkowsky, 1905) has fought a long uphill battle for acceptance. The main reason for reluctance among biologists to embrace the notion of endosymbiosis is probably because it runs contrary to Darwin's principle: endosymbiotic origins of organelles entail the occasional merger of two highly disparate cells into a single, bipartite genetic unit, simultaneously giving rise to novel and distinct taxa at higher levels (for example among the algae; Stoebe and Maier, 2002). Darwin envisaged nothing of the sort (but he was also not primarily concerned with microbes). Endosymbiotic models have always drawn support from modern, observable examples of symbioses between free-living cells; extrapolating back in time yields models of interspecific evolution, which can and must accommodate lateral gene transfer. Accepting the premise that cell–cell interactions similar to those observable today should also have occurred in the past (uniformitarianism) and drawing upon molecular data, biologists have gradually become accustomed to the view that chloroplasts and mitochondria were in fact once free-living prokaryotes (Sagan, 1967; Gray and Doolittle, 1982). Biochemical and molecular studies of modern symbioses will improve our understanding of the microbial past.

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