PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Evolutionary origins of metabolic compartmentalization in eukaryotes

William Martin

Phil. Trans. R. Soc. B 2010 **365**, 847-855
doi: 10.1098/rstb.2009.0252

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2010) **365**, 847–855
doi:10.1098/rstb.2009.0252

Review

Evolutionary origins of metabolic compartmentalization in eukaryotes

William Martin*

Institute of Botany III, University of Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany

Many genes in eukaryotes are acquisitions from the free-living antecedents of chloroplasts and mitochondria. But there is no evolutionary 'homing device' that automatically directs the protein product of a transferred gene back to the organelle of its provenance. Instead, the products of genes acquired from endosymbionts can explore all targeting possibilities within the cell. They often replace pre-existing host genes, or even whole pathways. But the transfer of an enzymatic pathway from one compartment to another poses severe problems: over evolutionary time, the enzymes of the pathway acquire their targeting signals for the new compartment individually, not in unison. Until the whole pathway is established in the new compartment, newly routed individual enzymes are useless, and their genes will be lost through mutation. Here it is suggested that pathways attain novel compartmentation variants via a 'minor mistargeting' mechanism. If protein targeting in eukaryotic cells possesses enough imperfection such that small amounts of entire pathways continuously enter novel compartments, selectable units of biochemical function would exist in new compartments, and the genes could become selected. Dual-targeting of proteins is indeed very common within eukaryotic cells, suggesting that targeting variation required for this minor mistargeting mechanism to operate exists in nature.

Keywords: endosymbiosis; chloroplasts; mitochondria; endosymbiotic gene transfer

1. INTRODUCTION

Metabolic compartmentation refers to the differential localization or non-homogeneous distribution of biochemical pathways within the cell and is founded in the differential localization of the enzymes involved. Although metabolic compartmentation is usually discussed in the context of eukaryotes, there are also cases of metabolic compartmentalization in prokaryotes. For example, the diffusion of proteins through the prokaryotic cytosol is slower than the diffusion of proteins through water (Elowitz *et al.* 1999) with the result that proteins expressed from the same location on a prokaryotic chromosome are, for a certain amount of time, located more closely to one another in space than proteins that are expressed from distant regions of the same chromosome. Because of that, if different subunits of a heteromeric enzyme are expressed next to each other on a prokaryotic chromosome, they will associate with each other to form an active enzyme faster than if they are expressed from distant loci. Similarly, products of enzymatic catalysis from the first enzyme in a metabolic pathway that is encoded by an operon will be more quickly converted to the next product by the second enzyme in the pathway if the second enzyme is encoded in the same polycistronic mRNA. Svetic *et al.* (2004) have suggested that even though this diffusion-limited compartmentalization through synthesis is temporally transient, it is precisely the selective advantage underlying operon formation in prokaryotes, and recent analyses of operons that have been independently assembled in disparate prokaryotic lineages are consistent with that view (Martin & McInerney 2009). That would be a general case of metabolic compartmentalization in prokaryotes that does not involve membranes, but rather hinges on the manifold consequences of cotranscriptional translation and molecular crowding in cells (Ellis & Minton 2006). It furthermore provides a compelling evolutionary rationale behind the origin of operons (Svetic *et al.* 2004; Martin & McInerney 2009).

There are also cases of membrane-bounded structures within prokaryotic cells that generate compartments, for example magnetosomes in the α-proteobacterium *Magnetospirillum magnetotacticum* (Komeili *et al.* 2006) or acidocalcisomes in the α-proteobacteria *Agrobacterium tumefaciens* (Seufferheld *et al.* 2003) and *Rhodospirillum rubrum* (Seufferheld *et al.* 2004), but it does not currently appear that multi-enzyme metabolic pathways are associated with such structures (Docampo *et al.* 2005, 2010). In that sense, those would be examples of membrane-bounded compartments that are not primarily involved in metabolism, but in storage and sequestering of inorganic compounds. Of course, the periplasmic space of Gram-negative bacteria is also a vast metabolic compartment (Abbott & Boraston 2008), but it is not located within the cytosol. The chromatophores of *Rhodobacter* and relatives (Geyer & Helms 2006), the

*w.martin@uni-duesseldorf.de

One contribution of 12 to a Theme Issue 'Evolution of organellar metabolism in unicellular eukaryotes'.

invaginated plasma membrane of many other proteobacteria, for example methanotrophs (Davies & Whittenbury 1970), and the thylakoid lumen of cyanobacteria can also be seen as metabolic compartments among prokaryotes, but the nature of those compartments has more to do with electron transport processes in the bioenergetic membranes bounding the compartments (Allen 2002) than with metabolic processes within the compartments themselves.

2. METABOLIC COMPARTMENTALIZATION IN EUKARYOTES: A RELIC OF ENDOSYMBIOSIS

Aside from a few such exceptions in prokaryotes, metabolic compartmentalization is an attribute specific to eukaryotic cells. Current understanding of metabolic compartmentalization in eukaryotes is founded in the endosymbiotic origin of organelles. Since the time 40 years ago that Lynn Margulis (as Sagan 1967) repopularized old but not completely forgotten (Ris & Plaut 1962) theories for the origin of chloroplasts (Mereschkowsky 1905) and mitochondria (Wallin 1927), biologists have generally viewed metabolic compartmentalization in eukaryotes in the light of endosymbiosis. Although there can be no question that the most complicated cases of cell compartmentalization in eukaryotes are found among the algae, with complex plastids acquired through secondary endosymbiosis (Stoebe & Maier 2002), this paper will focus on the simpler cases of eukaryotes harbouring only primary endosymbionts.

Chloroplasts were once free-living cyanobacteria that underwent an evolutionary transformation into metabolic compartments of oxygenic photosynthesis within eukaryotic cells (Gould *et al.* 2008; Lane & Archibald 2008; Kleine *et al.* 2009). Mitochondria were once free-living proteobacteria and have undergone an evolutionary transformation into metabolic compartments of oxygen-dependent and oxygen-independent pyruvate breakdown within eukaryotic cells (Tielens *et al.* 2002; van der Giezen 2009). Both organelle types have retained their own DNA, the sequence and structure of which attest beyond all reasonable doubt to the view that these organelles were once free-living prokaryotes. But the genomes of both organelles are very highly reduced relative to the genomes of their free-living cousins. Plastid genomes harbour anywhere from about 20 to about 200 protein-coding genes, mitochondrial genomes harbour between 3 and 63 genes (Timmis *et al.* 2004) or have relinquished their genome entirely in the case of mitosomes (Tovar *et al.* 2003; van der Giezen & Tovar 2005) and in the case of most, but not all, hydrogenosomes (Boxma *et al.* 2005; van der Giezen 2009).

Despite the genome reduction, fully functional chloroplasts and mitochondria contain approximately as many proteins as their free-living cousins, in the order of several thousand (Richly & Leister 2004). To explain the difference between the number of genes that organelle genomes harbour and the number of proteins that the organelles contain, there is a familiar corollary to endosymbiotic theory that was called endosymbiotic gene transfer (Martin *et al.* 1993): during the course of evolution, organelles relinquished many of their genes to the chromosomes of their host, where the genes were free to undergo recombination with host DNA in such a way as to acquire the expression and targeting signals that they required so that the encoded protein product could be translated on cytosolic ribosomes and imported into the organelle where its function could become selected. Once protein import of the host-encoded copy into the organelle is established, the organelle-encoded copy is freed from functional constraints and can be lost, thereby completing the process of gene relocation. Genes are not just transferred to the nucleus during endosymbiotic gene transfer, they are copied to the nucleus (Allen 2003) because a functional copy must remain intact in the organelle until a nuclear copy arises whose product can directly compete with the organelle copy.

Of course, the foregoing events of gene relocation and organelle targeting required that a protein import apparatus specific to the organelle in question was already in place. In the case of the chloroplast, one can readily imagine the situation at the very onset of endosymbiosis: the endosymbiont was still a fully fledged cyanobacterium, so if an endosymbiont lysed every so often, it would provide DNA that could be donated to the host. This kind of DNA transfer is commonly observed today from organelles to the nucleus (Timmis *et al.* 2004; Kleine *et al.* 2009) and very occasionally observed from bacterial endosymbionts to the nucleus (Hotopp *et al.* 2007). But before the protein import apparatus of the chloroplast—the TiC and ToC machinery encompassing dozens of proteins (Balsera *et al.* 2009)—had evolved, those cyanobacterium-derived genes that did become incorporated into the host's chromosomes and expressed there would either give rise to protein products targeted to the cytosol (acquisition of promoter only) or perhaps to pre-existing compartments like the mitochondrion (acquisition of a promoter and a pre-existing mitochondrial import signal). Because of this, the ancestor of plastids probably contributed to a significant extent to the complement of proteins in compartments other than the plastid (Martin *et al.* 2002).

The same sort of reasoning would apply if we consider the early phases of mitochondrial origin, where the endosymbiont was still a full-fledged proteobacterium. Before the origin of the mitochondrial protein import apparatus—the TiM and ToM machinery (Dolezal *et al.* 2006; Lithgow & Schneider 2010)—genes transferred from the mitochondrial endosymbiont to the host would have given rise to cytosolic proteins (Martin & Müller 1998) or proteins specific to other compartments pre-existing within the host cell prior to the origin of mitochondria.

From the foregoing, three facets to the evolution of compartmentalized metabolism in eukaryotes emerge that can be readily addressed as questions: (i) is there some inherent connection between a gene's evolutionary origin and the intracellular compartmentalization of its protein product? (ii) How can whole metabolic pathways be transferred from one

compartment to another? (iii) What sorts of pre-existing cell compartments did the host for the origin of mitochondria have before it acquired the mitochondrion, in other words, what kind of a cell was the host that acquired the mitochondrion? Each of the questions will be considered separately in the subsequent sections.

3. GENE ORIGIN AND PROTEIN COMPARTMENTALIZATION: IS THERE A CONNECTION?

The formulation of this question in its modern form can arguably be ascribed to Weeden (1981). He approached the problem from the fairly clear-cut perspective of chloroplasts: there are some plant proteins that occur as distinct isoenzymes in chloroplasts and the cytosol; the plastid genome is too small to encode all proteins in the plastid, hence the plant nuclear genes for the plastid-specific isoenzymes should ultimately come from cyanobacteria via gene transfer, while the cytosolic enzymes should be encoded by nuclear genes that reflect the ancestry of the host, and the nuclear-encoded chloroplast proteins have remained specific to their original compartment (Weeden called this the 'product specificity corollary'). By inference, 'all proteins specific to the plastid compartment are seen as those originally possessed by the postulated prokaryotic ancestor to the chloroplast' (Weeden 1981, p. 134). Other researchers might have seen the matter similarly at that time (Margulis 1981; Gray & Doolittle 1982), but few formulated the issue quite so crisply.

From the standpoint of someone who spent 10 years testing Weeden's product specificity corollary by separating chloroplast–cytosol isoenzymes common to the Calvin cycle and glycolysis in higher plants, purifiying and sequencing proteins, and isolating and sequencing the underlying genes to unravel their history through molecular evolution, it was surprising to see that although Weeden's hypothesis held up in the first molecular tests (Martin & Cerff 1986), the exceptions ultimately came to outnumber the rule. The bottom line of studies on about a dozen chloroplast–cytosol isoenzyme pairs was: there is no evolutionary 'homing device' that automatically directs the product of a transferred gene back to the organelle of its provenance, the products of genes that are acquired by endosymbionts are free to explore any and all targeting possibilities within the cell; they often can and do replace pre-existing host genes, or even whole pathways, and sometimes pre-existing host genes can be duplicated to provide organelle-targeted copies of host enzymes that can replace organelle-encoded functions (reviewed in Martin & Schnarrenberger 1997).

In addition to that survey, there are not very many other general theory sorts of papers on metabolic compartmentalization in the context of endosymbiosis beyond that of Weeden (1981). Borogard (2008) also picked up on this topic in about 2003 in what eventually became a posthumously published paper. He suggested that because of the greatness of evolutionary time since eukaryotes have existed, all variants for metabolic compartmentalization for any individual enzyme would have been explored within the cell, the implication being that selection will favour the most suitable biochemical environment for the operation of each individual enzyme. This scenario contains the same sort of 'exhaustive tinkering' component that can also be found in Ford Doolittle's earlier (1998) gene transfer ratchet model, where he suggested that 'all genes that can be replaced [...] will be, in the fullness of time' (Doolittle 1998). While Borogard's suggestion, like earlier considerations (Martin & Schnarrenberger 1997; Martin & Herrmann 1998), seems suitable to explain lineage-specific differences in the localization of individual enzymes, it does not provide a good solution to the problem of how to move entire pathways from one compartment to another, because in the evolution of pathways, the unit of selection is an entire series of substrate conversions, and one enzyme by itself in a new compartment is useless. Hence, even if some gene duplication occurred allowing one enzyme to acquire new targeting signals for access to a new compartment before it loses its function through mutation, the new variant would, in the vast majority of cases, be biochemically useless and hence unable to be fixed via selection. That brings us to the next section.

4. HOW CAN WHOLE PATHWAYS BE TRANSFERRED TO NEW COMPARTMENTS?

This question is anything but trivial, but was hardly ever given any serious attention outside the realm of research on specialized peroxisomes of kinetoplastids, glycosomes, that contain major segments of the glycolytic pathway (see the chapter by Ginger *et al.* 2010). The reason why it is a problem was just stated at the end of the foregoing section, but is more clearly worded by Michels & Opperdoes (1991) themselves: '...it is difficult to imagine how all the enzymes of an entire metabolic pathway could end up together in one organelle if they did not originate from it. Intermediate stages, with only some of the enzymes present in the organelle and others still in the cytosol, would not be an advantage but rather a burden to the cell.' This is one of the main reasons that glycosomes (Michels & Opperdoes 1991), and peroxisomes in general (de Duve 1969, 2007), were long considered to have originated from endosymbionts that were distinct from the ancestors of either mitochondria or plastids. It was simply very difficult to imagine how one could get a pathway from one compartment to another, one enzyme at a time. It was so difficult that endosymbiosis seemed to be the preferable explanation.

Thus, if we could find a simple evolutionary solution to the problem of how whole pathways move from one compartment to another, we would not need to resort to invoking endosymbiosis for the origin of free-living organelles that today are shown to stem from the endoplasmic reticulum (Platta & Erdmann 2007; Schrader & Yoon 2007; Tabak *et al.* 2008). What might such a solution be? If we consider the topogenic signals and molecular machinery that eukaryotes use to import proteins into membrane-bounded compartments, it is

clear that 100 per cent targeting specificity is hardly a realistic goal for a cell. If a protein is destined for the mitochondrion, a small amount (0.1–1% of the total activity) might end up in the peroxisome, or in the case of plants, a small amount might end up in the plastid. Keeping in mind that only a few per cent of wild-type enzyme activity is often sufficient to confer a wild-type phenotype with respect to growth (Stadler & Yanofsky 1959), the point is this: if a small amount of mistargeting is happening all the time in a eukaryotic cell, and if it is happening for all proteins of a pathway, then there could exist a small amount of whole pathways in incorrect compartments, more or less all the time. If so, that would provide a biochemically functioning unit of selection upon which standard evolutionary mechanisms of natural variation and natural selection could operate in such a way as to increase or decrease the amount of mistargeted pathway within the novel compartment. In principle, that would provide a plausible mechanism through which whole pathways could be redirected from one compartment to another during evolution. It would avoid the problem that re-targeting pathways one enzyme at a time is indeed an untenable prospect, as Michels & Opperdoes (1991) and Borst & Swinkels (1989) have pointed out, and it would not require endosymbiosis to explain every case of compartmentalized pathways.

That seems to be a satisfying idea, but is it realistic? To be tenable, this suggestion would demand that there is a biologically relevant amount of simultaneous protein-targeting to multiple compartments going on all the time in eukaryotic cells. How likely is it that to be true? If we look at the recent literature on the topic of dual-targeting, we see that it is very common indeed to see eukaryotic proteins directed to multiple compartments (Mackenzie 2005). Examples include targeting to mitochondria and chloroplasts (Hedtke *et al.* 1997; Duchene *et al.* 2005; Millar *et al.* 2006; Pino *et al.* 2007; Carrie *et al.* 2009), mitochondria, chloroplasts and the cytosol (Small *et al.* 1998), mitochondria and the endoplasmic reticulum (Bhagwat *et al.* 1999), mitochondria and the nucleus (Krause & Krupinska 2009), peroxisomes and mitochondria (Petrova *et al.* 2004), chloroplasts and the cytosol (Kiessling *et al.* 2004), hydrogenosomes and the cytosol (Mentel *et al.* 2008), and so forth. Dual-targeting is well known in fungi (Strobel *et al.* 2002), where mitochondrial and cytosolic localization can be shared for fumarase and aconitase for example (Regev-Rudzki *et al.* 2008, 2009), or mitochondrial, cytosolic and peroxisomal targeting can be found for isocitrate dehydrogenase (Szewczyk *et al.* 2001). Dual-targeting occurs in humans (Foster *et al.* 2006), in kinetoplastids (Hannaert *et al.* 1999) and there is generally a lot known about the underlying molecular mechanisms (Karniely & Pines 2005), although for the purposes of this paper, the specific nature of those mechanisms is not important, it is just important that they exist.

The list of known examples from the literature for multiple targeting of proteins within eukaryotic cells is much longer than those few mentioned here, and includes many more cases from plants, animals and fungi (Mitschke *et al.* 2009). In some cases, dual-targeting for specific proteins can be maintained in distinct lineages separated by more than 150 Myr of evolution (Morgante *et al.* 2009). Furthermore, dual-targeting has recently been shown to be an intermediate stage in the evolutionary replacement of a chloroplast-encoded ribosomal protein by a nuclear-encoded copy of a mitochondrial ribosomal protein during plant evolution (Ueda *et al.* 2008).

Thus, there seems to exist a considerable amount of slack in nature with respect to targeting specificity for a considerable number of proteins within a variety of eukaryotic cells, probably enough to allow the mistargeting model of pathway recompartmentalization to operate. Provided that there is also a corresponding amount of slack with respect to metabolite transport between compartments to provide substrates for low activities of mistargeted pathways to deliver products in new compartments, it is then not hard at all to see how new variants of metabolic compartmentalization might arise during evolution that could be immediately subjected to selection, fixation and refinement. New substrate distributions across compartments could be achieved either by relaxed substrate specificities of specifically targeted transporters or—to stick to only one mechanism—minor mistargeting of highly specific metabolite transporters. Neither seems hardly unreasonable. For the purpose of this argument, it is irrelevant whether the mechanism of dual-targeting (or mistargeting) involves alternative transcription initiation, alternative splicing, alternative start codons or post-translational modifications (Danpure 1995), or whether it involves ambiguous specificity during translocon–substrate interactions at the translocons themselves.

5. WHAT PRE-EXISTING CELL COMPARTMENTS DID THE HOST FOR THE ORIGIN OF MITOCHONDRIA POSSESS?

If we consider the evolution of metabolic compartmentalization in eukaryotes, there has to be a starting point somewhere, and that brings us to a perennially controversial topic, the origin of eukaryotes themselves. As recently outlined in more popular literature (Lane 2009; Zimmer 2009), there are two main opposing views on the origin of eukaryotes. They differ most notably with respect to the nature of the host that acquired the mitochondrion.

The traditional view posits that the host that acquired the mitochondrion was a full-fledged eukaryotic cell possessing a nucleus, an endoplasmic reticulum (ER), phagocytosis, flagella, cytoskeleton and essentially all eukaryotic traits with the exception of mitochondria, mitochondria being acquired by such a phagocytotic cell (Margulis *et al.* 2006; de Duve 2007; Cavalier-Smith 2009). That view has been around for about 40 years in various formulations stressing the endosymbiotic origin of flagella (Sagan 1967) or peroxisomes (de Duve 1969) at eukaryote origins. Above and beyond the lack of molecular evidence for an endosymbiotic origin of either flagella (Rizzotti 1995; Jekely & Arendt 2006) or peroxisomes (Gabaldón *et al.* 2006; Gabaldón 2010), a severely

nagging problem with the traditional view is that it predicts the existence of primitively amitochondriate eukaryotes, once called archezoa (Cavalier-Smith 1989). All groups ever thought to be archezoa (Patterson 1999) turned out to possess either anaerobic, H_2-producing forms of mitochondria called hydrogenosomes (Müller 2007; van der Giezen 2009), or to have highly reduced mitochondria called mitosomes (Tovar *et al.* 1999, 2003; Goldberg *et al.* 2008; Tsaousis *et al.* 2008; Hjort *et al.* 2010), pushing the origin of mitochondria back to the origin of eukaryotes themselves (Embley & Martin 2006).

The alternative view posits that the host that acquired the mitochondrion was a prokaryote, such that all eukaryote-specific compartments (mitochondria, the ER, the nucleus, peroxisomes) and eukaryotic-specific trait arose subsequently, in the wake of endosymbiosis. Some formulations of the prokaryote-host type of theory account for the common ancestry of mitochondria and hydrogenosomes (Martin & Müller 1998; van der Giezen 2009) and furthermore account for plausible selective forces underpinning the origin of the nucleus in the wake of mitochondrial origins (Martin & Koonin 2006). Newer molecular data and comparative genome analyses tend to favour prokaryote-host theories (Rivera & Lake 2004; Pisani *et al.* 2007; Cox *et al.* 2008; Koonin 2009), but there is currently no consensus as to the order of events concerning the origin of eukaryotic cell compartments. Proponents of the traditional models lament that prokaryote host models do not account for enough cell morphological details, while proponents of prokaryote host models lament that the traditional models fail to account for the observations in molecular data from genomes. For decades, traditional models have focused on phagocytosis and predation as the central, overarching and decisive evolutionary achievement in the prokaryote-to-eukaryote transition (Cavalier-Smith 1975, 2009), while overlooking the circumstance that the predatory lifestyle has indeed evolved on many different occasions among the prokaryotes, and each time it has brought forth highly effective predatory prokaryotic forms that invade, not engulf, their prey (Davidov & Jurkevitch 2009). One reason that phagocytosis was seen by some as so absolutely essential to the eukaryotic condition was based on the argument that without phagocytosis, it would not be possible for a prokaryote to take up residence within another cell (Cavalier-Smith 2002), but that argument is deflated by clear examples of prokaryotes living within other prokaryotes (Wujek 1979; von Dohlen *et al.* 2001).

Some critics of prokaryote host models argue that the assumed sisterhood of eukaryotes and archaebacteria as observed in some phylogenetic trees 'refutes all theories that eukaryotes originated by merging an archaebacterium and an α-proteobacterium' (Cavalier-Smith 2002), but more recent work indicates that the sisterhood of eukaryotes and archaebacteria seen in the most visible molecular phylogenies is a phylogeny reconstruction artefact, and that the archaebacterial-derived genes of eukaryotes stem from within the archaebacteria (Cox *et al.* 2008; Foster *et al.* 2009), rather than branching as a sister to them. Similar branching of eukaryotic informational genes from within the archaebacteria, as opposed to a sister group relationship, is also seen in supertree analyses (Pisani *et al.* 2007) and in other molecular analyses (Embley & Martin 2006).

6. CONCLUSION

In closing, one might also briefly consider a few other issues. For example there is the question of whether moonlighting, that is, the presence of different and distinct functions on the same polypeptide (Jeffery 2009), might bear upon the issue of pathway recompartmentalization, in that moonlighting proteins, if mistargeted, would carry more than one function to the new compartment, and this would allow for novel combinations of functions therein. But from the standpoint of recompartmentalizing core metabolic and biosynthetic pathways, the main enzymatic functions of the proteins involved, not other fortuitous properties thereof, stand in the foreground, hence moonlighting is more likely to assume a peripheral role in this context. There is also the issue of targeting proteins to fundamentally new compartments that are lacking altogether in prokaryotes like the ER lumen. But ER-targeting involves the sec pathway and the signal recognition particle, which are present in prokaryotes for targeting proteins to the plasma membrane (Albers *et al.* 2006; Rapoport 2007), such that not much in the way of fundamental molecular innovation was required to get proteins to the ER, once the cell has an ER, which is a different matter (Martin & Koonin 2006). Similarly, in secondary symbioses, the mechanisms of protein-targeting across the four membranes of many complex plastids are now rapidly being understood to involve surprisingly simple evolutionary recruitments of ER-derived protein-targeting components to direct proteins to a new organelle (Hempel *et al.* 2009; Spork *et al.* 2009). The secondary plastids provide fascinating examples of endosymbionts that bring along their own complete protein import machinery, the genes for which just have to become integrated into the chromosomes of the host, bringing us back to the general and widespread role of endosymbiotic gene transfer in cell evolution.

The arguments here embrace the premise that protein targeting in eukaryotes to compartments other than the cytosol is governed by information contained within the protein in question. There are reports that mRNA, rather than protein, might govern some protein-localization processes (Uniacke & Zerges 2009). While of interest, this neither favours nor disfavours the minor mistargeting model for pathway recompartmentalization. During the early evolution of eukaryotes, the transfer of pathways from the mitochondrial endosymbiont to the cytosol presents the simplest possible case: genes that are incorporated into the host's chromosomes can be expressed as cytosolic proteins without targeting information. This kind of biochemical reorganization in the wake of naturally occurring gene transfer in the context of endosymbiotic associations has been implicated in the origin of the eubacterial glycolytic pathway underpinning eukaryotic

energy metabolism (Martin & Müller 1998) and in the origin of the nuclear compartment itself (Martin & Koonin 2006). Recent phylogenetic findings support the view that the host for the origin of mitochondria stems from within the archaebacteria (Cox *et al.* 2008; Foster *et al.* 2009). It is thus all the more curious that not only the glycolytic pathway (Hannaert *et al.* 2000), but the vast majority, about 75 per cent, of eukaryotic genes possessing homologues in prokaryotes are more similar to eubacterial homologues than they are to archaebacterial homologues (Esser *et al.* 2004). But given the polarity of gene transfer from the ancestral mitochondrion—which appears to have been a metabolically quite versatile bacterium (Atteia *et al.* 2009)—and given the circumstance that genes were being transferred a chromosome's worth at a time, like happens today (Huang *et al.* 2005), if the first transfer does not lead to fixation, the next one might, and as long as there are at least two copies of the endosymbiont, the gene transfer ratchet is an inexhaustible source of new and potentially useful genes for a young but bipartite cell. How its expressed protein products came to be localized within their present compartments in various ensuing eukaryotic lineages appears to be up to natural variation and natural selection. However, for whole pathways to move, one protein at a time is not likely to get the job done, but a pinch of a whole pathway might.

Work in the author's laboratory is funded by the German Research Foundation and the European Research Council. I thank Giddy Landan and Tal Dagan for comments on the manuscript.

REFERENCES

Abbott, D. W. & Boraston, A. B. 2008 Structural biology of pectin degradation by Enterobacteriaceae. *Microbiol. Mol. Biol. Rev.* **72**, 301–316. (doi:10.1128/MMBR.00038-07)

Albers, S. V., Szabo, Z. & Driessen, A. J. M. 2006 Protein secretion in the Archaea: multiple paths towards a unique cell surface. *Nat. Rev. Microbiol.* **4**, 537–547. (doi:10.1038/nrmicro1440)

Allen, J. F. 2002 Photosynthesis of ATP—electrons, proton pumps, rotors, and poise. *Cell* **110**, 273–276. (doi:10.1016/S0092-8674(02)00870-X)

Allen, J. F. 2003 The function of genomes in bioenergetic organelles. *Phil. Trans. R. Soc. Lond. B* **358**, 19–37. (doi:10.1098/rstb.2002.1191)

Atteia, A. *et al.* 2009 A proteomic survey of *Chlamydomonas reinhardtii* mitochondria sheds new light on the metabolic plasticity of the organelle and on the nature of the alphaproteobacterial mitochondrial ancestor. *Mol. Biol. Evol.* **26**, 1533–1548. (doi:10.1093/molbev/msp068)

Balsera, M., Soll, J. & Bolter, B. 2009 Protein import machineries in endosymbiotic organelles. *Cell. Mol. Life Sci.* **66**, 1903–1923. (doi:10.1007/s00018-009-8644-2)

Bhagwat, S. V., Biswas, G., Anandatheerthavarada, H. K., Addya, S., Pandak, W. & Avadhani, N. G. 1999 Dual targeting property of the N-terminal signal sequence of P4501A1—targeting of heterologous proteins to endoplasmic reticulum and mitochondria. *J. Biol. Chem.* **274**, 24 014–24 022. (doi:10.1074/jbc.274.34.24014)

Borogard, L. 2008 Evolution of early eukaryotic cells: genomes, proteome and compartments. *Photosynth. Res.* **95**, 11–21.

Borst, P. & Swinkels, B. W. 1989 The evolutionary origin of glycosomes; how glycolysis moved from cytosol to organelle. In *Evolutionary tinkering in gene expression* (eds M. Grunberg-Manago, B. F. C. Clark & H. G. Zachau), pp. 163–174. New York, NY: Plenum Press.

Boxma, B. *et al.* 2005 An anaerobic mitochondrion that produces hydrogen. *Nature* **434**, 74–79. (doi:10.1038/nature03343)

Carrie, C., Giraud, E. & Whelan, J. 2009 Protein transport in organelles: dual targeting of proteins to mitochondria and chloroplasts. *FEBS J.* **276**, 1187–1195. (doi:10.1111/j.1742-4658.2009.06876.x)

Cavalier-Smith, T. 1975 The origin of nuclei and of eukaryotic cells. *Nature* **256**, 463–468. (doi:10.1038/256463a0)

Cavalier-Smith, T. 1989 Archaebacteria and Archezoa. *Nature* **339**, 100–101. (doi:10.1038/339100a0)

Cavalier-Smith, T. 2002 The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. *Int. J. Syst. Evol. Microbiol.* **52**, 297–354.

Cavalier-Smith, T. 2009 Predation and eukaryote cell origins: a coevolutionary perspective. *Int. J. Biochem. Cell Biol.* **41**, 307–322. (doi:10.1016/j.biocel.2008.10.002)

Cox, C. J., Foster, P. G., Hirt, R. P., Harris, S. R. & Embley, T. M. 2008 The archaebacterial origin of eukaryotes. *Proc. Natl Acad. Sci. USA* **105**, 20356–20361. (doi:10.1073/pnas.0810647105)

Danpure, C. J. 1995 How can the products of a single gene be localized to more than one intracellular compartment? *Trends Cell Biol.* **5**, 230–238. (doi:10.1016/S0962-8924(00)89016-9)

Davidov, Y. & Jurkevitch, E. 2009 Predation between prokaryotes and the origin of eukaryotes. *BioEssays* **31**, 748–757. (doi:10.1002/bies.200900018)

Davies, S. L. & Whittenbury, R. 1970 Fine structure of methane and other hydrocarbon-utilizing bacteria. *J. Gen. Microbiol.* **61**, 227–232.

de Duve, C. 1969 Evolution of the peroxisome. *Ann. NY Acad. Sci.* **168**, 369–381.

de Duve, C. 2007 The origin of eukaryotes: a reappraisal. *Nat. Rev. Genet.* **8**, 395–403. (doi:10.1038/nrg2071)

Docampo, R., de Souza, W., Miranda, K., Rohloff, P. & Moreno, S. N. J. 2005 Acidocalcisomes—conserved from bacteria to man. *Nat. Rev. Microbiol.* **3**, 251–261. (doi:10.1038/nrmicro1097)

Docampo, R., Ulrich, P. & Moreno, S. N. J. 2010 Evolution of acidocalcisomes and their role in polyphosphate storage and osmoregulation in eukaryotic microbes. *Phil. Trans. R. Soc. B* **365**, 775–784. (doi:10.1098/rstb.2009.0179)

Dolezal, P., Likic, V., Tachezy, J. & Lithgow, T. 2006 Evolution of the molecular machines for protein import into mitochondria. *Science* **313**, 314–318. (doi:10.1126/science.1127895)

Doolittle, W. F. 1998 You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. *Trend. Genet.* **14**, 307–311.

Duchene, A. M., Giritch, A., Hoffmann, B., Cognat, V., Lancelin, D., Peeters, N. M., Zaepfel, M., Marechal-Drouard, L. & Small, I. D. 2005 Dual targeting is the rule for organellar aminoacyl-tRNA synthetases in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **102**, 16 484–16 489. (doi:10.1073/pnas.0504682102)

Ellis, R. J. & Minton, A. P. 2006 Protein aggregation in crowded environments. *Biol. Chem.* **387**, 485–497. (doi:10.1515/BC.2006.064)

Elowitz, M. B., Surette, M. G., Wolf, P. E., Stock, J. B. & Leibler, S. 1999 Protein mobility in the cytoplasm of *Escherichia coli*. *J. Bact.* **181**, 197–203.

Embley, T. M. & Martin, W. 2006 Eukaryotic evolution, changes and challenges. *Nature* **440**, 623–630. (doi:10.1038/nature04546)

Esser, C. *et al.* 2004 A genome phylogeny for mitochondria among α-proteobacteria and a predominantly eubacterial ancestry of yeast nuclear genes. *Mol. Biol. Evol.* **21**, 1643–1660. (doi:10.1093/molbev/msh160)

Foster, L. J., de Hoog, C. L., Zhang, Y., Zhang, Y., Xie, X., Mootha, V. K. & Mann, M. 2006 A mammalian organelle map by protein correlation profiling. *Cell* **125**, 187–199. (doi:10.1016/j.cell.2006.03.022)

Foster, P. G., Cox, C. J. & Embley, T. M. 2009 The primary divisions of life: a phylogenomic approach employing composition-heterogeneous methods. *Phil. Trans. R. Soc. B* **364**, 2197–2207. (doi:10.1098/rstb.2009.0034)

Gabaldón, T. 2010 Peroxisome diversity and evolution. *Phil. Trans. R. Soc. B* **365**, 765–773. (doi:10.1098/rstb.2009.0240)

Gabaldón, T., Snel, B., van Zimmeren, F., Hemrika, W., Tabak, H. & Huynen, M. A. 2006 Origin and evolution of the peroxisomal proteome. *Biol. Direct* **1**, Article No. 8. (doi:10.1186/1745-6150-1-8)

Geyer, T. & Helms, V. 2006 A spatial model of the chromatophore vesicles of *Rhodobacter sphaeroides* and the position of the cytochrome bc_1 complex. *Biophys. J.* **91**, 921–926. (doi:10.1529/biophysj.105.078501)

Ginger, M. L., McFadden, G. I. & Michels, P. A. M. 2010 Re-wiring and regulation of cross-compartmentalized metabolism in protists. *Phil. Trans. R. Soc. B* **365**, 831–845. (doi:10.1098/rstb.2009.0259)

Goldberg, A. V. *et al.* 2008 Localization and functionality of microsporidian iron–sulphur cluster assembly proteins. *Nature* **452**, 624–628. (doi:10.1038/nature06606)

Gould, S. B., Waller, R. R. & McFadden, G. I. 2008 Plastid evolution. *Annu. Rev. Plant. Biol.* **59**, 491–517. (doi:10.1146/annurev.arplant.59.032607.092915)

Gray, M. W. & Doolittle, W. F. 1982 Has the endosymbiont hypothesis been proven? *Microbol. Rev.* **46**, 1–42.

Hannaert, V., Adjé, C. A., de Walque, S. & Michels, P. A. M. 1999 Compartmentation of glycolytic enzymes in Kinetoplastida. *Res. Rev. Parasitol.* **59**, 85–100.

Hannaert, V. *et al.* 2000 Enolase from *Trypanosoma brucei*, from the amitochondriate protist *Mastigamoeba balamuthi*, and from the chloroplast and cytosol of *Euglena gracilis*: pieces in the evolutionary puzzle of the eukaryotic glycolytic pathway. *Mol. Biol. Evol.* **17**, 989–1000.

Hedtke, B., Börner, T. & Weihe, A. 1997 Mitochondrial and chloroplast phage-type RNA polymerases in *Arabidopsis*. *Science* **277**, 809–811. (doi:10.1126/science.277.5327.809)

Hempel, F., Bullmann, L., Lau, J., Zauner, S. & Maier, U. G. 2009 ERAD-derived preprotein transport across the second outermost plastid membrane of diatoms. *Mol. Biol. Evol.* **26**, 1781–1790. (doi:10.1093/molbev/msp079)

Hjort, K., Goldberg, A. V., Tsaousis, A. D., Hirt, R. P. & Embley, T. M. 2010 Diversity and reductive evolution of mitochondria among microbial eukaryotes. *Phil. Trans. R. Soc. B* **365**, 713–727. (doi:10.1098/rstb.2009.0224)

Hotopp, J. C. D. *et al.* 2007 Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. *Science* **317**, 1753–1756. (doi:10.1126/science.1142490)

Huang, C. Y., Grünheit, N., Ahmadinejad, N., Timmis, J. N. & Martin, W. 2005 Mutational decay and age of chloroplast and mitochondrial genomes transferred recently to angiosperm nuclear chromosomes. *Plant Physiol.* **138**, 1723–1733. (doi:10.1104/pp.105.060327)

Jeffery, C. J. 2009 Moonlighting proteins—an update. *Mol. Biosyst.* **5**, 345–350. (doi:10.1039/b900658n)

Jekely, G. & Arendt, D. 2006 Evolution of intraflagellar transport from coated vesicles and autogenous origin of the eukaryotic cilium. *BioEssays* **28**, 191–198. (doi:10.1002/bies.20369)

Karniely, S. & Pines, O. 2005 Single translation–dual destination: mechanisms of dual protein targeting in eukaryotes. *EMBO Rep.* **6**, 420–425. (doi:10.1038/sj.embor.7400394)

Kiessling, J., Martin, A., Gremillon, L., Rensing, S. A., Nick, P., Sarnighausen, E., Decker, E. L. & Reski, R. 2004 Dual targeting of plastid division protein FtsZ to chloroplasts and the cytoplasm. *EMBO Rep.* **5**, 889–894. (doi:10.1038/sj.embor.7400238)

Kleine, T., Maier, U. -G. & Leister, D. 2009 DNA transfer from organelles to the nucleus: the idiosyncratic genetics of endosymbiosis. *Annu. Rev. Plant Biol.* **60**, 115–138. (doi:10.1146/annurev.arplant.043008.092119)

Komeili, A., Li, Z., Newman, D. K. & Jensen, G. J. 2006 Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science* **311**, 242–245. (doi:10.1126/science.1123231)

Koonin, E. V. 2009 Darwinian evolution in the light of genomics. *Nucleic Acids Res.* **37**, 1011–1034. (doi:10.1093/nar/gkp089)

Krause, K. & Krupinska, K. 2009 Nuclear regulators with a second home in organelles. *Trends Plant Sci.* **14**, 194–199. (doi:10.1016/j.tplants.2009.01.005)

Lane, N. 2009 *Life ascending: the ten great inventions of evolution*, p. 344. London, UK: Profile Books Ltd.

Lane, C. E. & Archibald, J. M. 2008 The eukaryotic tree of life: endosymbiosis takes its TOL. *Trends. Ecol. Evol.* **23**, 268–275. (doi:10.1016/j.tree.2008.02.004)

Lithgow, T. & Schneider, A. 2010 Evolution of macromolecular import pathways in mitochondria, hydrogenosomes and mitosomes. *Phil. Trans. R. Soc. B* **365**, , 799–817. (doi:10.1098/rstb.2009.0167)

Mackenzie, S. A. 2005 Plant organellar protein targeting: a traffic plan still under construction. *Trends Cell Biol.* **15**, 548–554. (doi:10.1016/j.tcb.2005.08.007)

Margulis, L. 1981 *Symbiosis in cell evolution*, p. 419. San Francisco, CA: Freeman.

Margulis, L., Chapman, M., Guerrero, R. & Hall, J. 2006 The last eukaryotic common ancestor (LECA): acquisition of cytoskeletal motility from aerotolerant spirochetes in the Proterozoic Eon. *Proc. Natl Acad. Sci. USA* **103**, 13 080–13 085. (doi:10.1073/pnas.0604985103)

Martin, W. & Cerff, R. 1986 Prokaryotic features of a nucleus encoded enzyme: cDNA sequences for chloroplast and cytosolyic glyceraldehyde-3-phosphate dehydrogenases from mustard (*Sinapis alba*). *Eur. J. Biochem.* **159**, 323–331. (doi:10.1111/j.1432-1033.1986.tb09871.x)

Martin, W. & Herrmann, R. G. 1998 Gene transfer from organelles to the nucleus: how much, what happens and why? *Plant Physiol.* **118**, 9–17. (doi:10.1104/pp.118.1.9)

Martin, W. & Koonin, E. V. 2006 Introns and the origin of nucleus–cytosol compartmentalization. *Nature* **440**, 41–45. (doi:10.1038/nature04531)

Martin, F. J. & McInerney, J. O. 2009 Recurring cluster and operon assembly for phenylacetate degradation genes. *BMC Evol. Biol.* **9**, 36. (doi:10.1186/1471-2148-9-36)

Martin, W. & Müller, M. 1998 The hydrogen hypothesis for the first eukaryote. *Nature* **392**, 37–41. (doi:10.1038/32096)

Martin, W. & Schnarrenberger, C. 1997 The evolution of the Calvin cycle from prokaryotic to eukaryotic chromosomes: a case study of functional redundancy in ancient pathways through endosymbiosis. *Curr. Genet.* **32**, 1–18. (doi:10.1007/s002940050241)

Martin, W., Brinkmann, H., Savona, C. & Cerff, R. 1993 Evidence for a chimaeric nature of nuclear genomes:

eubacterial origin of eukaryotic glyceraldehyde-3-phosphate dehydrogenase genes. *Proc. Natl Acad. Sci. USA* **90**, 8692–8696. (doi:10.1073/pnas.90.18.8692)

Martin, W. *et al.* 2002 Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *Proc. Natl Acad. Sci. USA* **99**, 12 246–12 251. (doi:10.1073/pnas.182432999)

Mentel, M., Zimorski, V., Haferkamp, P., Martin, W. & Henze, K. 2008 Protein import into hydrogenosomes of *Trichomonas vaginalis* involves both N-terminal and internal targeting signals: a case study of thioredoxin reductases. *Eukaryot. Cell* **7**, 1750–1757. (doi:10.1128/EC.00206-08)

Mereschkowsky, C. 1905 Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. *Biol. Centralbl.* **25**, 593–604. [English translation in Martin, W. & Kowallik, K. V. 1999 *Eur. J. Phycol.* **34**, 287–295.]

Michels, P. A. M. & Opperdoes, F. R. 1991 The evolutionary origin of glycosomes. *Parasitol. Today* **7**, 105–109. (doi:10.1016/0169-4758(91)90167-M)

Millar, A. H., Whelan, J. & Small, I. 2006 Recent surprises in protein targeting to mitochondria and plastids. *Curr. Opin. Plant Biol.* **9**, 610–615. (doi:10.1016/j.pbi.2006.09.002)

Mitschke, J., Fuss, J., Blum, T., Hoglund, A., Reski, R., Kohlbacher, O. & Rensing, S. A. 2009 Prediction of dual protein targeting to plant organelles. *New Phytol.* **183**, 224–236. (doi:10.1111/j.1469-8137.2009.02832.x)

Morgante, C. V., Rodrigues, R. A. O., Marbach, P. A. S., Borgonovi, C. M., Moura, D. S. & Silva-Filho, M. C. 2009 Conservation of dual-targeted proteins in *Arabidopsis* and rice points to a similar pattern of gene-family evolution. *Mol. Genet. Genom.* **281**, 525–538. (doi:10.1007/s00438-009-0429-7)

Müller, M. 2007 The road to hydrogenosomes. In *Origin of mitochondria and hydrogenosomes* (eds W. F. Martin & M. Müller), pp. 1–12. Heidelberg, Germany: Springer-Verlag.

Patterson, D. J. 1999 The diversity of eukaryotes. *Am. Nat.* **65**, S96–S124.

Petrova, V. Y., Drescher, D., Kujumdzieva, A. V. & Schmitt, M. J. 2004 Dual targeting of yeast catalase A to peroxisomes and mitochondria. *Biochem. J.* **380**, 393–400. (doi:10.1042/BJ20040042)

Pino, P., Foth, B. J., Kwok, L. Y., Sheiner, L., Schepers, R., Soldati, T. & Soldati-Favre, D. 2007 Dual targeting of antioxidant and metabolic enzymes to the mitochondrion and the apicoplast of *Toxoplasma gondii*. *PLoS Pathogens* **3**, 1092–1108.

Pisani, D., Cotton, J. A. & McInerney, J. O. 2007 Supertrees disentangle the chimerical origin of eukaryotic genomes. *Mol. Biol. Evol.* **24**, 1752–1760. (doi:10.1093/molbev/msm095)

Platta, H. W. & Erdmann, R. 2007 The peroxisomal protein import machinery. *FEBS Lett.* **581**, 2811–2819. (doi:10.1016/j.febslet.2007.04.001)

Rapoport, T. A. 2007 Protein translocation across the eukaryotic endoplasmic reticulum and bacterial plasma membranes. *Nature* **450**, 663–669. (doi:10.1038/nature06384)

Regev-Rudzki, N., Yogev, O. & Pines, O. 2008 The mitochondrial targeting sequence tilts the balance between mitochondrial and cytosolic dual localization. *J. Cell Sci.* **121**, 2423–2431. (doi:10.1242/jcs.029207)

Regev-Rudzki, N., Battat, E., Goldberg, I. & Pines, O. 2009 Dual localization of fumarase is dependent on the integrity of the glyoxylate shunt. *Mol. Microbiol.* **72**, 297–306. (doi:10.1111/j.1365-2958.2009.06659.x)

Richly, E. & Leister, D. 2004 An improved prediction of chloroplast proteins reveals diversities and commonalities in the chloroplast proteomes of *Arabidopsis* and rice. *Gene* **329**, 11–16. (doi:10.1016/j.gene.2004.01.008)

Ris, H. & Plaut, W. 1962 Ultrastructure of DNA-containing areas in the chloroplasts of *Chlamydomonas*. *J. Cell Biol.* **12**, 383–391.

Rivera, M. C. & Lake, J. A. 2004 The ring of life provides evidence for a genome fusion origin of eukaryotes. *Nature* **431**, 152–155. (doi:10.1038/nature02848)

Rizzotti, M. 1995 Cilium: origin and 9-fold symmetry. *Acta Biotheoretica* **43**, 227–240. (doi:10.1007/BF00707271)

Sagan, L. 1967 On the origin of mitosing cells. *J. Theoret. Biol.* **14**, 225–274. (doi:10.1016/0022-5193(67)90079-3)

Schrader, M. & Yoon, Y. 2007 Mitochondria and peroxisomes: are the 'Big Brother' and the 'Little Sister' closer than assumed? *BioEssays* **29**, 1105–1114. (doi:10.1002/bies.20659)

Seufferheld, M., Vieira, M. C. F., Ruiz, F. A., Rodrigues, C. O., Moreno, S. N. J. & Docampo, R. 2003 Identification of organelles in bacteria similar to acidocalcisomes of unicellular eukaryotes. *J. Biol. Chem.* **278**, 29 971–29 978. (doi:10.1074/jbc.M304548200)

Seufferheld, M., Lea, C. R., Vieira, M., Oldfield, E. & Docampo, R. 2004 The $H^{(+)}$-pyrophosphatase of *Rhodospirillum rubrum* is predominantly located in polyphosphate-rich acidocalcisomes. *J. Biol. Chem.* **279**, 51 193–51 202. (doi:10.1074/jbc.M406099200)

Small, I., Wintz, H., Akashi, K. & Mireau, H. 1998 Two birds with one stone: genes that encode products targeted to two or more compartments. *Plant Mol. Biol.* **38**, 265–277. (doi:10.1023/A:1006081903354)

Spork, S., Hiss, J. A., Mandel, K., Sommer, M., Kooij, T. W. A., Chu, T., Schneider, G., Maier, U. G. & Przyborski, J. M. 2009 An unusual ERAD-like complex is targeted to the apicoplast of *Plasmodium falciparum*. *Euk. Cell* **8**, 1134–1145. (doi:10.1128/EC.00083-09)

Stadler, J. & Yanofsky, C. 1959 Studies on a series of tryptophan-independent strains derived from a tryptophan-requiring mutant of *Escherichia coli*. *Genetics* **44**, 105–123.

Stoebe, B. & Maier, G. 2002 One, two, three: nature's toolbox for building plastids. *Protoplasma* **219**, 123–130. (doi:10.1007/s007090200013)

Strobel, G., Zollner, A., Angermayr, M. & Bandlow, W. 2002 Competition of spontaneous protein folding and mitochondrial import causes dual subcellular location of major adenylate kinase. *Mol. Biol. Cell* **13**, 1439–1448. (doi:10.1091/mbc.01-08-0396)

Svetic, R. E., MacCluer, C. R., Buckley, C. O., Smythe, K. L. & Jackson, J. H. 2004 A metabolic force for gene clustering. *Bull. Mathemat. Biol.* **66**, 559–581. (doi:10.1016/j.bulm.2003.09.008)

Szewczyk, E., Andrianopoulos, A., Davis, M. A. & Hynes, M. J. 2001 A single gene produces mitochondrial, cytoplasmic, and peroxisomal NADP-dependent isocitrate dehydrogenase in *Aspergillus nidulans*. *J. Biol. Chem.* **276**, 37 722–37 729. (doi:10.1074/jbc.M105645200)

Tabak, H. F., van der Zand, A. & Braakman, I. 2008 Peroxisomes: minted by the ER. *Curr. Opin. Cell Biol.* **20**, 393–400. (doi:10.1016/j.ceb.2008.05.008)

Tielens, A. G. M., Rotte, C., van Hellemond, J. J. & Martin, W. 2002 Mitochondria as we don't know them. *Trends Biochem. Sci.* **27**, 564–572. (doi:10.1016/S0968-0004(02)02193-X)

Timmis, J. N., Ayliffe, M. A., Huang, C. Y. & Martin, W. 2004 Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. *Nat. Rev. Genet.* **5**, 123–135. (doi:10.1038/nrg1271)

Tovar, J., Fischer, A. & Clark, C. G. 1999 The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. *Mol. Microbiol.* **32**, 1013–1021. (doi:10.1046/j.1365-2958.1999.01414.x)

Tovar, J., León-Avila, G., Sánchez, L. B., Sutak, R., Tachezy, J., van der Giezen, M., Hernández, M., Müller, M. & Lucocq, J. M. 2003 Mitochondrial remnant organelles of *Giardia* function in iron–sulphur protein maturation. *Nature* **426**, 172–176. (doi:10.1038/nature01945)

Tsaousis, A. D., Kunji, E. R. S., Goldberg, A. V., Lucocq, J. M., Hirt, R. P. & Embley, T. M. 2008 A novel route for ATP acquisition by the remnant mitochondria of *Encephalitozoon cuniculi*. *Nature* **453**, 553–556. (doi:10.1038/nature06903)

Ueda, M., Nishikawa, T., Fujimoto, M., Takanashi, H., Arimura, S., Tsutsumi, N. & Kadowaki, K. 2008 Substitution of the gene for chloroplast RPS16 was assisted by generation of a dual targeting signal. *Mol. Biol. Evol.* **25**, 1566–1575. (doi:10.1093/molbev/msn102)

Uniacke, J. & Zerges, W. 2009 Chloroplast protein targeting involves localized translation in *Chlamydomonas*. *Proc. Natl Acad. Sci. USA* **106**, 1439–1444. (doi:10.1073/pnas.0811268106)

van der Giezen, M. 2009 Hydrogenosomes and mitosomes: conservation and evolution of functions. *J. Eukaryot. Microbiol.* **56**, 221–231. (doi:10.1111/j.1550-7408.2009.00407.x)

van der Giezen, M. & Tovar, J. 2005 Degenerate mitochondria. *EMBO Rep.* **6**, 525–530. (doi:10.1038/sj.embor.7400440)

von Dohlen, C. D., Köhler, S., Alsop, S. T. & McManus, W. R. 2001 Mealybug β-proteobacterial symbionts contain γ-proteobacterial symbionts. *Nature* **412**, 433–436. (doi:10.1038/35086563)

Wallin, I. E. 1927 *Symbionticism and the origin of species*, p. 171. London, UK: Bailliere, Tindall and Cox.

Weeden, N. F. 1981 Genetic and biochemical implications of the endosymbiotic origin of the chloroplast. *J. Mol. Evol.* **17**, 133–139. (doi:10.1007/BF01733906)

Wujek, D. E. 1979 Intracellular bacteria in the blue-green-alga *Pleurocapsa minor*. *Trans. Am. Micros. Soc.* **98**, 143–145. (doi:10.2307/3225953)

Zimmer, C. 2009 On the origin of eukaryotes. *Science* **325**, 666–668. (doi:10.1126/science.325_666)