PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The evolution of organellar metabolism in unicellular eukaryotes

Michael L. Ginger, Geoffrey I. McFadden and Paul A. M. Michels

Phil. Trans. R. Soc. B 2010 **365**, 693-698
doi: 10.1098/rstb.2009.0260

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2010) **365**, 693–698
doi:10.1098/rstb.2009.0260

Introduction

The evolution of organellar metabolism in unicellular eukaryotes

Michael L. Ginger[1,*], Geoffrey I. McFadden[2] and Paul A. M. Michels[3]

[1]*School of Health and Medicine, Division of Biomedical and Life Sciences, Lancaster University, Lancaster LA1 4YQ, UK*
[2]*Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria 3010, Australia*
[3]*Research Unit for Tropical Diseases, de Duve Institute, and Laboratory of Biochemistry, Université Catholique de Louvain, TROP 74.39, Avenue Hippocrate 74, B-1200 Brussels, Belgium*

Metabolic innovation has facilitated the radiation of microbes into almost every niche environment on the Earth, and over geological time scales transformed the planet on which we live. A notable example of innovation is the evolution of oxygenic photosynthesis which was a prelude to the gradual transformation of an anoxic Earth into a world with oxygenated oceans and an oxygen-rich atmosphere capable of supporting complex multicellular organisms. The influence of microbial innovation on the Earth's history and the timing of pivotal events have been addressed in other recent themed editions of *Philosophical Transactions of Royal Society B* (Cavalier-Smith *et al.* 2006; Bendall *et al.* 2008). In this issue, our contributors provide a timely history of metabolic innovation and adaptation within unicellular eukaryotes. In eukaryotes, diverse metabolic portfolios are compartmentalized across multiple membrane-bounded compartments (or organelles). However, as a consequence of pathway retargeting, organelle degeneration or novel endosymbiotic associations, the metabolic repertoires of protists often differ extensively from classic textbook descriptions of intermediary metabolism. These differences are often important in the context of niche adaptation or the structure of microbial communities. Fundamentally interesting in its own right, the biochemical, cell biological and phylogenomic investigation of organellar metabolism also has wider relevance. For instance, in some pathogens, notably those causing some of the most significant tropical diseases, including malaria, unusual organellar metabolism provides important new drug targets. Moreover, the study of organellar metabolism in protists continues to provide critical insight into our understanding of eukaryotic evolution.

Keywords: adaptation; cellular metabolism; endosymbiosis; organellar genomes; organellogenesis; protein targeting

1. A POLARIZED VIEW OF ORGANELLAR METABOLISM

Many of the pioneering discoveries in metabolism and key principles in enzymology that are the bedrock of most general biochemistry textbooks and many biochemistry classes were made or established prior to the emergence of molecular biology as a discipline in the late 1960s. Some even pre-date the widespread application of electron microscopy and emergence of cell biology as a discipline in the 1940s and 1950s. Little wonder perhaps that within one of the *Reflections* and *Classics* commissioned to celebrate the centenary of the *Journal of Biological Chemistry* in 2005, Hanson (2005: 1705) remarked.

> I have spent a lifetime in the area of intermediary metabolism and have seen it move from the center of biochemistry to the backwater of our science in a very short period, only to be re-discovered by a new generation of biologists. By 1970, the writing was on the wall for metabolism; it was largely considered a 'mature area', lacking excitement; molecular biology was the area of the future.

Of course, basic studies of human and plant metabolism continue to be important areas of research in the life sciences—the prevalence of obesity and diabetes as chronic health concerns and the issue of food provision for a global population, which is increasing in size, provide prime illustrations of areas where contemporary

* Authors for correspondence (m.ginger@lancaster.ac.uk, gim@unimelb.au (GIM), paul.michels@uclouvain.be (PAMM)).

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

metabolic research is vibrant. Yet, it is probably also true that to many the compartmentalization of the ubiquitous core reactions in intermediary metabolism is a backwater, where the key discoveries, such as the characterization of cytosolic carbohydrate catabolism, the mitochondrial metabolism of pyruvate and the oxidation of acetyl-CoA through the tricarboxylic acid (or Krebs) cycle, the principles of chemiosmotic theory and oxidative phosphorylation or the role of chloroplasts in carbon fixation, oxygen production and photorespiration, were made long ago. However, in contrast to genetically tractable or easily culturable species, such as the bakers' yeast *Saccharomyces cerevisiae* or the green alga *Chlamydomonas reinhardtii*, many unicellular eukaryotes had until recently been subjected to comparatively few metabolic studies. Within the last decade the complete genetic blueprints for many important parasitic and free-living eukaryotic microbes were deciphered, suddenly providing quite detailed metabolic maps for a diverse array of protists and fungi that had previously been experimentally challenging or little studied. Coupled to careful cytology and other molecular studies, the comparative analysis of these genomes has underpinned a dramatic reappraisal of metabolic compartmentalization in eukaryotes: case histories of unexpected secondary, and even tertiary, endosymbioses have been identified, the reductive evolution (or degeneracy) of organellar function has been observed, and unprecedented novelties and flexibility have even been added to already complex pictures of cross-compartmentalized metabolism in the better-studied unicellular eukaryotes, such as *C. reinhardtii* (e.g. Atteia *et al.* 2006; Dubini *et al.* 2009). Most significantly, perhaps, work during the last 15 years has fundamentally changed our view of eukaryotic evolution. Organisms such as *Giardia lamblia* or *Entamoeba histolytica* are therefore no longer recognized as amitochondriate taxa that evolved prior to the endosymbiosis that gave rise to mitochondria. Instead, we now know that double-membrane-bounded organelles of mitochondrial ancestry can be found in all extant eukaryotes examined thus far (e.g. Hjort *et al.* 2010; Lithgow & Schneider 2010). Indeed, the endosymbiotic association with the α-proteobacterium that evolved into the proto-mitochondrion is widely held to have been a driving force in eukaryogenesis *per se* though there is vigorous debate as to how, when and why (e.g. Martin & Müller 1998; Embley & Martin 2006; Martin & Koonin 2006; de Duve 2007; Cavalier-Smith 2009 for further discussion). Doubtless, the debate on eukaryotic origins will continue for some years to come, although the topic is not considered at length in any of the articles published here. Below, we highlight three broad areas that we felt a themed discussion on 'the evolution of organellar metabolism in unicellular eukaryotes' should encompass and trail the expert contributions that were solicited. We hope readers will agree that each expert contribution, while providing an independent discussion in its own right, also contributes to a progressive narrative. A current view of the likely evolutionary relationships between the many diverse unicellular eukaryotes discussed in these contributions is summarized in figure 1.

2. FUNCTIONAL DIVERSITY WITHIN ORGANELLES

Within the papers from the first half of this theme, the focus is very clearly on the functional diversity seen in different types of organelles. Our authors chart evolutionary histories for, and discuss the biochemical diversity within, organelles of endosymbiotic origin (mitochondria and plastids), organelles that are the product of eukaryotic innovation (peroxisomes) and organelles that are present in both eukaryotes and prokaryotes (acidocalcisomes). The acquisition of mitochondria and chloroplasts are widely viewed as the most significant examples of endosymbiosis in cellular evolution, but a fascinating and diverse array of transient and stable endosymbiotic relationships is found in evolutionarily diverse eukaryotes. These endosymbiotic relationships confer novel metabolic functions to the host and, in some instances, are likely to be informative for understanding many aspects of the organellogenesis that underpinned the evolution of early eukaryotic cells. Thus, the first contribution to the evolution of organellar metabolism in unicellular eukaryotes is the discussion by Nowack & Melkonian (2010) of endosymbiotic associations within protists. Their wide-ranging text not only illustrates the ubiquity of diverse endosymbioses within many ecologically significant settings, but also explores two endosymbioses, in *Paulinella chromatophora* and *Hatena arenicola*, respectively, that provide the reader with examples where close regulation, potentially even cell-cycle entrainment, of host–endosymbiont interactions are informing on the endosymbiont-to-organelle transition. Indeed, Nowack and Melkonian discuss the evidence that the chromatophore of *P. chromatophora* represents a second independent origin for primary plastids, a possibility that is also discussed by Keeling (2010) in his contribution on plastid diversity. Finally, *Myrionecta rubra* and various dinoflagellate species provide the reader with curious examples of host cells that recurrently thieve some of the organelles (karyoklepty and kleptoplastidy) from their ingested eukaryotic prey.

The next three contributions focus on the modification and moderation of mitochondria and chloroplasts. Hjort *et al.* (2010) discuss diversity and reductive evolution of mitochondria, bringing together a wealth of data from the last 15 or so years that led to the realization that all known extant eukaryotes possess double-membrane-bounded organelles of mitochondrial descent, including organisms such as *G. lamblia* or *E. histolytica*, which had been widely thought to represent primitive, amitochondriate taxa. This reductive evolution results either in the evolution of mitochondria that lack the capacity for oxidative phosphorylation but produce ATP from substrate-level phosphorylations and make hydrogen (hydrogenosomes), or miniature, morphologically simple mitochondria called mitosomes, which lack any capacity for organellar-free energy generation. Mitochondrial degeneracy has occurred independently within most of the major eukaryotic groups, and mitosomes have been found in both anaerobes and some aerobic parasites. In addition to ascribing functions to the mitosomes from different eukaryotes,

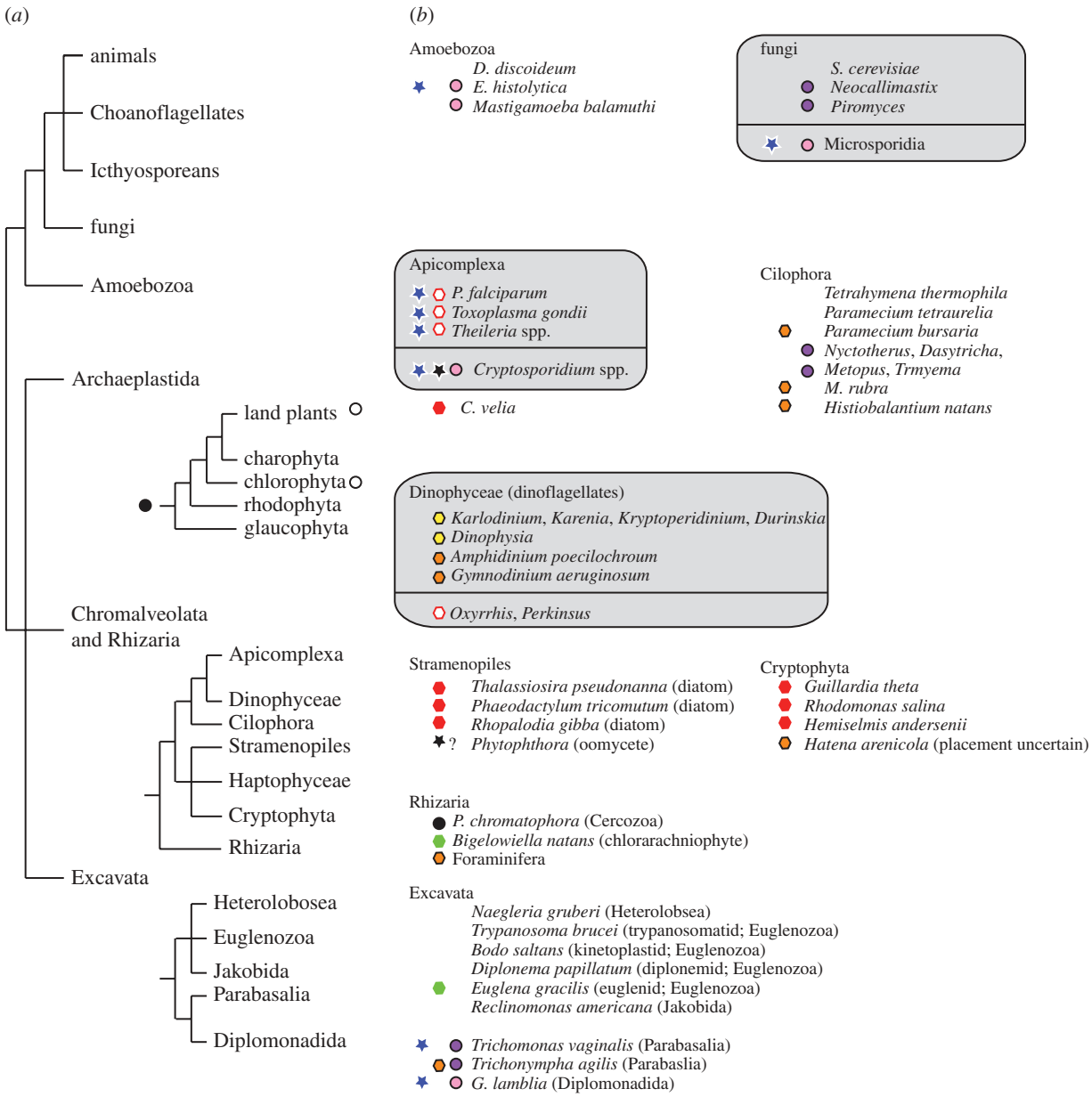

Figure 1. Eukaryotic phylogeny and diverse organellar metabolism. (*a*) Relationships between eukaryotic supergroups (note: the animals, choanoflagellates, icthyosporeans and fungi are collectively known as the opisthokonts). Likely relationships within supergroups of the key groups discussed in many of the articles published in this themed edition are also shown. These putative relationships represent consensus views of recent datasets (Embley & Martin 2006; Rodriguez-Ezpeleta *et al.* 2007; Burki *et al.* 2008; Lee *et al.* 2008; Hampl *et al.* 2009; Roger & Simpson 2009). (*b*) Some of the key taxa discussed in articles published in this theme are stated. The symbols give some indication as to the unusual organellar metabolism that is covered elsewhere in this issue. Black circles and hexagons with white interiors denote non-photosynthetic plastids of either primary or secondary origin, respectively. Taxa drawn beneath lines (e.g. *Cryptosporidium*) represent basal or early-diverging taxa within the stated group. *Chromera velia* is shown as the closest known relative to the Apicomplexa, although it is not included within this phylum (Moore *et al.* 2008). Purple circle, mitochondria-like organelles/hydrogenosomes; pink circle, mitosomes; black circle, 1° plastid; red hexagon, 2° red plastid; green hexagon, 2° green plastid; yellow hexagon, 3° plastid; black star, plastid loss; blue star, peroxisome loss; orange hexagons, discussed in the context of an unusual endosymbiotic relationship.

another key question is how, having dispensed with the capacity for organellar energy production, these mitochondria acquire the ATP they require for the ATP-dependent processes of protein import and Fe–S cluster assembly that still occur within the organelle matrix. Hjort *et al.* point out that some organisms have solved this problem using atypical ADP/ATP carriers or even bacterial-type nucleotide transporters to support ATP import into mitosomes, but they also remind us that how ATP is acquired in some species (e.g. *G. lamblia*) is not at all obvious.

In contrast to mitochondria, where diversity is 'merely' a consequence of reductive evolution, plastid diversity is the legacy of serial endosymbioses, organelle displacement and reductive evolution that has extended in at least one instance to the point of organelle loss. Keeling (2010) provides an overview of this topic and the key areas of debate that have either been

informed by, or resulted from, the molecular data published in the last decade. Lim & McFadden (2010), in contrast, focus on one particular example of reductive evolution in plastids. They discuss the biology of the vestigial plastid, or apicoplast, that is present in most parasites of the phylum Apicomplexa. Malarial parasites belong to the Apicomplexa, and the discovery of a relic non-photosynthetic plastid in these organisms during the 1990s was not only totally unexpected, but suggested new prospects for the development of drugs against *Plasmodium falciparum*, the species responsible for the majority of malarial deaths. Lim and McFadden debate the origin of the Apicomplexa, discuss the mechanisms by which proteins are targeted to the apicoplast and examine the biochemical functions of the organelle. The new directions in which the current research is moving, such as the progress towards identifying the membrane protein inventory of the apicoplast, and some of the outstanding questions that remain regarding apicoplast function are all discussed. Significantly, in their address of function, the authors make use of recent data to shift the emphasis from the description of metabolic maps to a more detailed biochemical consideration of redox and energy balance within the organelle. This changing emphasis is particularly important given the translational possibility of targeting apicoplast function for drug design.

In the remaining articles that focus exclusively on the biology of different organelles, the attention switches to organelles that did not evolve through endosymbiosis. Gabaldón (2010) discusses the evolutionary plasticity of peroxisomes, which are organelles that lack DNA and are bounded by a single membrane. Approximately 30 proteins are known to be involved in peroxisome biogenesis and the insertion of at least some membrane proteins is dependent upon prior routing through the endoplasmic reticulum. Although classically associated with the formation and detoxification of H_2O_2 and lipid metabolism, Gabaldón (2010) discusses how modern comparative analyses have revealed considerable metabolic diversity within the peroxisomes of different species. Again, just as with plastids, there are examples of organelle loss within evolutionarily diverse eukaryotes, but another striking feature that is relevant in an evolutionary context is the flexibility and rapidity with which the enzyme content of these organelles can be remodelled. There is much still left to learn about peroxisomal cell biology and biochemistry in most microbial eukaryotes, but the extensive remodelling of peroxisomes that occurs, for instance, in methylotrophic yeasts or trypanosomes in response to environmental change or during cell differentiation, as well as the unique compartmentalization of numerous enzymes of carbohydrate metabolism within trypanosome peroxisomes (see also the contributions by Ginger *et al.* (2010) and Martin (2010)), suggests that considerable hidden diversity awaits discovery.

Finally, acidocalcisomes provide a rare example of organellar biology common to both prokaryotes and eukaryotes. Docampo *et al.* (2010) speculate that the storage of phosphate and cations represents the ancestral functions of these organelles with critical, dynamic roles in cation and pH homeostasis and osmoregulation representing functions that emerged in eukaryotic cells. Like the recently emerged view of peroxisome biogenesis, new data also suggest that in eukaryotes protein trafficking leans on processes and proteins involved in endomembrane trafficking, too.

3. ORGANELLE BIOGENESIS AND TURNOVER

The texts by Gabaldón (2010) and Docampo *et al.* (2010) illustrate the dynamic nature of organelle structure and function; the text on peroxisomes describes how enzyme content within organelles changes rapidly in response to developmental or environmental cues, and in the example of acidocalcisomes fusion with the contractile vacuole, and dynamic changes in cation content or polyphosphate mobilization are critical for function. Thus, in the middle section of this theme, we commissioned three articles that spoke directly to very different aspects of organelle biogenesis or turnover in an evolutionary context.

First, the narrative returns to mitochondria and chloroplasts, where protein content and composition is the product of both organellar and nuclear gene expression. Barbrook *et al.* (2010) provide a wide-ranging overview of genome structure, gene content and gene expression and control from mitochondrial and plastid genomes. Here, content refers not only to the number and identities of protein-coding genes, but also the organization of rRNA genes and the presence or absence of tRNA genes. Moreover, in some species, gene expression requires the *trans*-splicing of partial open-reading frames or transcript editing in order to produce translatable mRNA species. An important message that comes across in the text from Barbrook *et al.* is the staggering diversity that exists with regard to the structure, organization, content and post-transcriptional modifications found in protist mitochondria. Although such diversity in content and organization is less apparent in chloroplast genomes, Barbrook *et al.* discuss how studies with chloroplasts have provided the greater insight, to date, into how redox poise and ATP/ADP ratio can be important determinants for gene expression from organellar genomes. The authors close their discussion by reference to the nucleomorphs (or miniaturized, relic nuclei) that are retained between the inner two and outer chloroplast membranes of the independently acquired secondary plastids in chlorarachniophyte and cryptophyte algae; the extent to which known mechanisms of gene regulation have been retained in these tiny nuclei or the potential for nucleomorph gene expression to respond to either nuclear or chloroplast signals is not known.

Overviews or summaries of the relevant protein import pathways are provided in articles written by Hjort *et al.*, Keeling, Lim and McFadden, and Gabaldón, but Lithgow & Schneider (2010) write extensively on the evolution of macromolecular import pathways in mitochondria, hydrogenosomes and mitosomes. Here, the authors not only consider the evolution of the canonical mitochondrial import pathways that have been dissected at the molecular level in *S. cerevisiae* and the divergence from these

import pathways in protists and other fungi, but extend their discussion of macromolecular import to tRNA species, too. The suggestion that tRNAs were imported into mitochondria was initially viewed with some doubt, and then thought to be limited to only a few taxa (in their text Barbrook *et al*. also highlight how not all eukaryotes retain full or even partial sets of tRNA genes on the mitochondrial genome). Although the current understanding of tRNA import machineries is still fragmentary, the process is in fact widespread and requires first the delivery of tRNA to mitochondria, and then import of tRNA into the mitochondrial matrix. There is currently no evidence for a dedicated tRNA import apparatus in any eukaryote, but consistent with the idea that mitochondrial tRNA import evolved on multiple occasions during eukaryotic evolution, Lithgow and Schneider discuss how (i) the mechanism of tRNA translocation across mitochondrial membranes appears not to be conserved between different taxa and (ii) different housekeeping proteins have been co-opted to participate in the process of tRNA delivery to mitochondria in the organisms studied to date.

The process of organelle turnover is considered in the paper by Kiel (2010) who discusses the molecular mechanisms of autophagy, the process by which cells recycle intracellular components by targeting to the vacuole/lysosome. The term 'autophagy', first coined by Christian de Duve in 1963, covers a multitude of selective and non-selective degradation pathways, which are used to target organelles or cytosol to lysosomes in response to either nutritional or developmental cues. However, the ongoing molecular dissection of autophagy pathways, achieved largely as a consequence of the tractable genetics available for *S. cerevisiae* and other yeasts, has occurred only during the last 20 years, and the process of autophagy is recently known to be important in many aspects of development and human health. Drawing on the data obtained by studies with yeast models, Kiel gives an overview of the major autophagy pathways, before turning his discussion to the social amoeba *Dictyostelium discoideum* and pathogenic parasites of the protist genera *Leishmania*, *Trypanosoma* and *Entamoeba*, where autophagy is critical for differentiation into niche-adapted morphologies. Importantly, Kiel uses his discussion and supplementary information to illustrate how the basic form of autophagy is conserved across all eukaryotes. Thus, to some observers previously published work documenting the absence from the fore-mentioned parasites of easily recognizable Atg5-Atg10-Atg12-Atg16-dependent ubiquitin-like conjugation systems might have pointed towards the presence of more primitive, ancestral-like autophagy pathways in some eukaryotes not closely related to well-studied yeast models. However, Kiel reveals that the apparent absence is more likely to reflect divergence of autophagy systems, rather than the primary absence or moderation of canonical autophagy. In contrast, although at least one example of organelle-selective autophagy, called pexophagy, is also conserved between evolutionarily distant taxa, the proteins involved in the initiation/regulation of organelle-selective autophagy are not well conserved between different taxa.

4. RETOOLING ORGANELLES AND SOME ASSOCIATED CONSEQUENCES OF RECOMPARTMENTALIZATION

We close the theme with two contributions where the authors consider aspects of organellar metabolism and its evolution that are not specifically addressed elsewhere. In the first of these articles, the editors turn to examples where the compartmentalization of core metabolic pathways has been subject to reorganization in various unicellular eukaryotes. In particular, the authors use the example of isoprenoid biosynthesis to illustrate how plastid acquisition and loss, as well as niche adaptation, can influence the overall organization of cellular metabolism and describe how glycolysis, perhaps the best known and most central of all the metabolic pathways, has been subject to unusual recompartmentalization in several lineages. In the case of glycolysis, the selective drivers that resulted in the re-routing of metabolic pathways into novel organellar locations within the stramenopiles and trypanosomatids are not obvious but, as with the recompartmentalization of any metabolic pathway, bring the inevitable consequence of needing to retune regulatory networks and mechanisms. In the case of the glycolytic enzymes that were moved behind the peroxisomal membranes in a common ancestor of the parasitic trypanosomatids and free-living kinetoplastids, the effect on regulatory retuning is relatively well understood. This knowledge is reviewed by Ginger *et al.* (2010), although the authors' principal aim is to use the intriguing and evolutionarily relevant example of trypanosome glycolysis to showcase how the modern approaches of mathematical modelling and systems biology can be used to uncover or understand novel modes of pathway regulation. As with the example of apicoplast function (Lim & McFadden 2010), the study of trypanosome glycolysis has possible medical relevance for drug design, since several trypanosomatid species are also the etiologic agents of serious tropical diseases.

In the final article of our theme issue, Martin (2010) addresses what is probably the most generic question with regard to the origin and evolution of organellar metabolism in eukaryotes. Namely, how are intact metabolic pathways either targeted back to their organelles of origin, in the case of mitochondria and chloroplasts that evolve from endosymbioses (and from which most of the original genome was lost or transferred to the host's nucleus), or routed into new cellular locations? The magnitude of the problem is succinctly phrased by Martin (2010):

> individual enzymes will acquire their targeting signals for [a] new compartment one at a time, not in unison. Yet not until the whole pathway is established [in that new compartment] the newly routed individual enzymes are useless, hence their genes cannot come under [. . . .] positive selection and therefore [can] be lost through mutation.

To deal with this problem, Martin proposes that a low degree of constitutive mistargeting allows multiple enzymes from individual metabolic pathways to arrive continually into new cellular compartments, and thence potentially come under purifying selection if

misrouting is biochemically favourable. To support this provocative, but appealing argument, Martin draws upon observations that a few per cent of individual enzyme activity is often sufficient to maintain the wild-type phenotype with respect to growth and the numerous examples of dual targeting that have been reported for proteins in evolutionarily diverse taxa.

We would like to express our sincere thanks to all the contributing authors for their willingness to produce timely articles for this themed edition. We also thank Claire Rawlinson, James Joseph and the rest of the editorial team of *Philosophical Transactions of Royal Society B* for the enjoyable opportunity to put this volume together. M.L.G. acknowledges the support of a Royal Society University Research Fellowship. G.I.M. is supported by an ARC Federation Fellowship, a Howard Hughes Medical Institute International Scholars Award and a programme grant from the NHMRC. P.A.M.M. acknowledges support from the 'Fonds de la Recherche Scientifique Médicale' and the Belgian Interuniversity Attraction Poles—Federal Office for Scientific, Technical and Cultural Affairs.

REFERENCES

Atteia, A., van Lis, R., Gelius-Dietrich, G., Adrait, A., Garin, J., Joyard, J., Rolland, N. & Martin, W. 2006 Pyruvate formate-lyase and a novel route of eukaryotic ATP synthesis in *Chlamydomonas* mitochondria. *J. Biol. Chem.* **281**, 9909–9918. (doi:10.1074/jbc.M507862200)

Barbrook, A. C., Howe, C. J., Kurniawan, D. P. & Tarr, S. J. 2010 Organisation and expression of organellar genomes. *Phil. Trans. R. Soc. B* **365**, 785–797. (doi:10.1098/rstb.2009.0250)

Bendall, D. S., Howe, C. J., Nisbet, E. G. & Nisbet, R. E. 2008 Photosynthetic and atmospheric evolution. Introduction. *Phil. Trans. R. Soc. B* **363**, 2625–2628. (doi:10.1098/rstb.2008.0058)

Burki, F., Shalchian-Tabrizi, K. & Pawlowski, J. 2008 Phylogenomics reveals a new 'megagroup' including most photosynthetic eukaryotes. *Biol. Lett.* **4**, 366–369. (doi:10.1098/rsbl.2008.0224)

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)

Cavalier-Smith, T., Brasier, M. & Embley, T. M. 2006 Introduction: how and when did microbes change the world? *Phil. Trans. R. Soc. B* **361**, 845–850. (doi:10.1098/rstb.2006.1847)

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

Docampo, R., Ulrich, J. & 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)

Dubini, A., Mus, F., Seibert, M., Grossman, A. R. & Posewitz, M. C. 2009 Flexibility in anaerobic metabolism as revealed in a mutant of *Chlamydomonas reinhardtii* lacking hydrogenase activity. *J. Biol. Chem.* **284**, 7201–7213. (doi:10.1074/jbc.M803917200)

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

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

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

Hampl, V., Hug, L., Leigh, J. W., Dacks, J. B., Lang, B. F., Simpson, A. G. & Roger, A. J. 2009 Phylogenomic analyses support the monophyly of Excavata and resolve relationships among eukaryotic 'supergroups'. *Proc. Natl Acad. Sci. USA* **106**, 3859–3864. (doi:10.1073/pnas.0807880106)

Hanson, R. W. 2005 Metabolism in the era of molecular biology. *J. Biol. Chem.* **280**, 1705–1715. (doi:10.1074/jbc.X400009200)

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)

Keeling, P. J. 2010 The endosymbiotic origin, diversification, and fate of plastids. *Phil. Trans. R. Soc. B* **365**, 729–748. (doi:10.1098/rstb.2009.0103)

Kiel, J. A. K. W. 2010 Autophagy in unicellular eukaryotes. *Phil. Trans. R. Soc. B* **365**, 819–830. (doi:10.1098/rstb.2009.0237)

Lee, S. C., Corradi, N., Byrnes III, E. J., Torres-Martinez, S., Dietrich, F. S., Keeling, P. J. & Heitman, J. 2008 Microsporidia evolved from ancestral sexual fungi. *Curr. Biol.* **18**, 1675–1679. (doi:10.1016/j.cub.2008.09.030)

Lim, L. & McFadden, G. I. 2010 The evolution, metabolism and functions of the apicoplast. *Phil. Trans. R. Soc. B* **365**, 749–763. (doi:10.1098/rstb.2009.0273)

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)

Martin, W. 2010 Evolutionary origins of metabolic compartmentalization in eukaryotes. *Phil. Trans. R. Soc. B* **365**, 847–855. (doi:10.1098/rstb.2009.0252)

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

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

Moore, R. B. *et al.* 2008 A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* **451**, 959–963. (doi:10.1038/nature06635)

Nowack, E. & Melkonian, M. 2010 Endosymbiotic associations within protists. *Phil. Trans. R. Soc. B* **365**, 699–712. (doi:10.1098/rstb.2009.0188)

Rodriguez-Ezpeleta, N., Brinkmann, H., Burger, G., Roger, A. J., Gray, M. W., Philippe, H. & Lang, B. F. 2007 Toward resolving the eukaryotic tree: the phylogenetic positions of jakobids and cercozoans. *Curr. Biol.* **17**, 1420–1425. (doi:10.1016/j.cub.2007.07.036)

Roger, A. J. & Simpson, A. G. 2009 Evolution: revisiting the root of the eukaryote tree. *Curr. Biol.* **19**, R165–R167. (doi:10.1016/j.cub.2008.12.032)