Research Focus

The missing link between hydrogenosomes and mitochondria

William Martin

Institut für Botanik III, Heinrich-Heine Universität Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany

Mitochondria typically respire oxygen and possess a small DNA genome. But among various groups of oxygen-shunning eukaryotes, typical mitochondria are often lacking, organelles called hydrogenosomes being found instead. Like mitochondria, hydrogenosomes are surrounded by a double-membrane, produce ATP and sometimes even have cristae. In contrast to mitochondria, hydrogenosomes produce molecular hydrogen through fermentations, lack cytochromes and usually lack DNA. Hydrogenosomes do not fit into the conceptual mold cast by the classical endosymbiont hypothesis about the nature of mitochondria. Accordingly, ideas about their evolutionary origins have focussed on the differences between the two organelles instead of their commonalities. Are hydrogenosomes fundamentally different from mitochondria, the result of a different endosymbiosis? Or are our concepts about the mitochondrial archetype simply too narrow? A new report has uncovered DNA in the hydrogenosomes of anaerobic ciliates. The sequences show that these hydrogenosomes are, without a doubt, mitochondria in the evolutionary sense, even though they differ from typical mitochondria in various biochemical properties. The new findings are a benchmark for our understanding of hydrogenosome origins.

Introduction

Ciliates are an extremely diverse group of protists [1], particularly when it comes to their dependence on oxygen [2]. Aerobic, mitochondrion-bearing lineages interleave in molecular phylogenies with anaerobic, hydrogenosome-bearing organisms, indicating that specializations to aerobic and/or anaerobic environments have occurred several times independently in ciliate evolution [3]. Such findings have led to the notion that hydrogensomes might be forms of mitochondria that are specialized to function in low oxygen environments [4]. However, to date, in studies of the ciliates, as with other eukaryotic groups [4], either mitochondria or hydrogenosomes have been found, never any intermediates. Therefore, one might ask, where are the missing links that might connect the aerobic and anaerobic forms? New findings by Brigitte Boxma *et al.* [5] reveal that the first bona fide intermediate organelles that unite the hallmark features of mitochondria (a DNA genome) and hydrogenosomes (H_2-production) are found in the anaerobic ciliate Nyctotherus ovalis, which inhabits the cockroach hindgut.

The mitochondria of *N. ovalis* that Boxma *et al.* [5] describe have rather well-defined cristae. In contrast to the mammalian mitochondria – the ones that are depicted in standard biochemistry textbooks – the mitochondria of *Nyctotherus* do not transfer the electrons from glucose oxidation to O_2 as the terminal electron acceptor of a mitochondrial respiratory chain. Hence, they do not produce H_2O as a major end product of core energy metabolism [6]. Instead, *Nyctotherus* performs fermentations, donating a portion of the electrons from glucose oxidation to small, endogenously generated organic compounds, such as pyruvate and fumarate (yielding, for example, lactate and succinate as excreted end products); another portion of the electrons is transferred to protons, yielding molecular hydrogen (H_2) as a major metabolic end product [6].

Various hydrogenosome-bearing ciliates harbour methanogenic endosymbionts, which conduct interspecies hydrogen transfer with the organelles and are sometimes also physically associated with them [2,3]. In the case of *Nyctotherus*, this is a sensitive bioassay indicating that the organelles do indeed produce H_2 [5], upon which the methanogenic endosymbionts depend for their growth and survival. This physiological interaction (anaerobic syntrophy) is widespread in anaerobic habitats [2,3]. In some anaerobic ciliates, the methanogens even undergo dramatic morphological changes during physical association with hydrogenosomes [3]; this is due to the H_2-dependence of the former and the H_2-production of the latter. The *Nyctotherus* mitochondria are designated as hydrogenosomes because they produce H_2, the defining characteristic of the organelle [7,8]. In addition to the ciliates, several other groups of eukaryotes are also known to have hydrogenosomes [9], including the parabasalids (where hydrogenosomes were discovered [7]) and the chytridiomycete fungi [10].

Bridging the gap

The hydrogenosomes (the H_2-producing mitochondria) of *Nyctotherus* are unique in that they – unlike any hydrogenosomes studied so far – contain their own DNA [5]. This DNA appears to be a rather typical ciliate mitochondrial genome like those found among aerobic ciliates: it encodes ribosomal proteins and components of the mitochondrial respiratory chain [5], which is a significant finding. Since the mid-1990s, evidence has

Corresponding author: Martin, W. (w.martin@uni-duesseldorf.de).
Available online 16 August 2005

accumulated to suggest that hydrogenosomes and mitochondria are aerobic and anaerobic manifestations of one and the same organelle [9] but there were still some skeptics [11,12]. This was because the anaerobic ATP-producing nature of hydrogenosomes, and the anaerobic ATP-producing pathways in mitochondria in general, have never meshed with conventional views of mitochondrial evolution, which account mainly for O_2-dependent functions of the organelle at mitochondrial origins [12]. The findings of Boxma *et al.* [5], in addition to the recent findings of Hrdy *et al.* [13] concerning the evolutionary origin of trichomonad hydrogenosomes [6–8], strengthens the evidence for common ancestry of mitochondria and hydrogenosomes significantly.

Furthermore, the *Nyctotherus* hydrogenosomes share attributes not only with O_2-respiring mitochondria (the genome) but also with mitochondria that produce ATP without the need for oxygen but that do not produce H_2 – organelles designated as anaerobic mitochondria [14]. Similar to the anaerobic mitochondria of some metazoans, the mitochondrion of *Nyctotherus* possesses an incomplete citric acid cycle [5]. This was shown in feeding experiments with glucose uniformly labelled with ^{14}C and glucose labelled with ^{14}C only at atom C6. Were a complete citric acid cycle operating, as in most aerobic mitochondria, C6-labelled glucose would have released radioactive CO_2 due to the operation of multiple rounds of the citric acid cycle. *Nyctotherus* released no $^{14}CO_2$ from C6-labelled glucose. Instead, substantial amounts of the ^{14}C label were excreted as acetate and succinate, end products that are typical of anaerobic mitochondria because they occur in protists and various metazoans that, for part or all of their life cycle, are exposed to anoxic environments [14]. The detection of radioactively labelled succinate is significant because it indicates that endogenously produced fumarate is serving as a terminal electron acceptor in *Nyctotherus*. Fumarate reduction in anaerobic mitochondria usually requires the presence of rhodoquinone with its stronger reducing potential [14] rather than ubiquinone (typical of aerobic mitochondria). In line with that view, Boxma *et al.* [5] detected rhodoquinone in *N. ovails*, albeit at relatively low amounts, leaving open an interesting issue as to its functional significance.

Finally, Boxma *et al.* [5] also report several expressed sequence tags from *Nyctotherus* that indicate the presence of typically mitochondrial functions in these hydrogenosomes. Inhibitor studies suggest the presence of functional complex I and complex II in the mitochondrion along with the lack of a terminal oxidase, while also confirming that *N. ovalis* is indeed an anaerobe because prolonged exposure to O_2 kills the organism. Like most anaerobes, it can survive short-term oxygen exposure because it possesses biochemical mechanisms to remove O_2 that do not involve a respiratory chain. Overall, the data of Boxma *et al.* [5] leave no doubt that the *Nyctotherus* organelles are hydrogenosomes with a mitochondrial genome. There is no sensible evolutionary interpretation of the findings of this group other than the idea they express; namely that they have uncovered a hitherto missing transitional state in the evolutionary sequence relating mitochondria and hydrogenosomes within the ciliates and, by inference, within other eukaryotic groups as well.

Why such a fuss?

Why is anaerobic biochemistry in a hydrogenosome with a genome significant? Because it sits squarely on a weakness of the various manifestations of the endosymbiont hypothesis that the literature has to offer, into which anaerobic eukaryotes have never really fit snugly [2,4,6–8,12,14] and because it fulfills a crucial prediction of a competing alternative [15]. Assuming that mitochondria and hydrogenosomes share a single eubacterial common ancestor that was capable of producing ATP either with or without the help of oxygen, and hence assuming that anaerobic specializations of that endosymbiotic organelle (hydrogenosomes) occurred independently in different eukaryotic lineages, intermediate forms should be discovered that unite the attributes of both organelle types. Boxma *et al.* [5] report such an intermediate.

Evolutionary biology thrives on debate. Those who view a single common ancestry of hydrogenosomes and mitochondria with skepticism [11,12,16] might caution that the lack of DNA in hydrogenosomes from other eukaryotic groups would not exclude the possibility of recurrent hydrogenosome origins from independent endosymbionts. However, common mitochondrial ancestry from a single eubacterial progenitor [4–6,9,13–15] is currently the null hypothesis entailing the fewest corrollary assumptions. Furthermore, alternative ideas about hydrogenosomal origins that would directly account for the presence of a mitochondrial genome in Nyctotherus hydrogenosomes have not been articulated so far. Indeed, if DNA in the organelle is the only evidence that would count in court on this evolutionary issue, the burden of proof to reject common ancestry would require the discovery of hydrogenosomes whose DNA reflects ancestry from a prokaryote that was unequivocally distinct from the mitochondrial symbiont. Until then, Occam's razor would weigh in favour of the simplest among competing alternatives while hydrogenosomes continue to uncover the surprising life of anaerobic eukaryotes.

References

1 Lynn, D.H. (2003) Morphology or molecules: How do we identify the major lineages of ciliates (Phylum Ciliophora)? *Eur. J. Protistol.* 39, 356–364
2 Fenchel, T. and Finlay, B.J. (1995) *Ecology and Evolution in Anoxic Worlds*, Oxford Series in Ecology and Evolution, Oxford University Press
3 Embley, T.M. and Finlay, B.J. (1994) The use of small subunit rRNA sequences to unravel the relationships between anaerobic ciliates and their methanogen endosymbionts. *Microbiology* 140, 225–235
4 Embley, T.M. *et al.* (1997) Anaerobic eukaryote evolution: hydrogenosomes as biochemically modified mitochondria? *Trends Ecol. Evol.* 12, 437–441
5 Boxma, B. *et al.* (2005) An anaerobic mitochondrion that produces hydrogen. *Nature* 434, 74–79
6 Müller, M. (2003) Energy metabolism. Part I: Anaerobic protozoa. In *Molecular Medical Parasitology* (Marr, J., ed.), pp. 125–139, Academic Press
7 Lindmark, D.G. and Müller, M. (1973) Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate, *Tritrichomonas foetus* and its role in pyruvate metabolism. *J. Biol. Chem.* 248, 7724–7728

8 Müller, M. (1993) The hydrogenosome. *J. Gen. Microbiol.* 139, 2879–2889
9 Embley, T.M. *et al*. (2003) Hydrogenosomes, mitochondria and early eukaryotic evolution. *IUBMB Life* 55, 387–395
10 Yarlett, N. *et al*. (1986) Hydrogenosomes in the rumen fungus *Neocallimastix patriciarum*. *Biochem. J.* 236, 729–739
11 Dyall, S.D. *et al*. (2004) Non-mitochondrial complex I proteins in a hydrogenosomal oxidoreductase complex. *Nature* 431, 1103–1107
12 Andersson, S.G.E. and Kurland, C.G. (1999) Origins of mitochondria and hydrogenosomes. *Curr. Opin. Microbiol.* 2, 535–541
13 Hrdy, I. *et al*. (2004) *Trichomonas* hydrogenosomes contain the NADH dehydrogenase module of mitochondrial complex I. *Nature* 432, 618–622
14 Tielens, A.G.M. *et al*. (2002) Mitochondria as we don't know them. *Trends Biochem. Sci.* 27, 564–572
15 Martin, W. and Müller, M. (1998) The hydrogen hypothesis for the first eukaryote. *Nature* 392, 37–41
16 Margulis, L. *et al*. (2000) The chimeric eukaryote: origin of the nucleus from the karyomastigont in amitochondriate protists. *Proc. Natl. Acad. Sci. U. S. A.* 97, 6954–6959

0966-842X/$ - see front matter
doi:10.1016/j.tim.2005.08.005

Cell individuality: the bistability of competence development

Simon V. Avery

School of Biology, Institute of Genetics, University of Nottingham, University Park, Nottingham NG7 2RD, UK

Heterogeneous development of competence among cells of the bacterium *Bacillus subtilis* provides an appealing model of cell individuality, an area that is currently attracting considerable research interest. Under appropriate conditions, only a fraction of cells in an isogenic culture become competent for transformation. Two experimental studies have now pinpointed the same auto-stimulatory feedback loop of gene expression as the principal requirement for the establishment of this 'bistable' response.

Phenotypic heterogeneity

Would a world in which all organisms of a population behaved in the same 'optimized' way necessarily be a better one? Evidently not. Phenotypic diversity is manifest in all organisms and in microorganisms it is readily apparent among individual cells even within isogenic populations. The benefits of such non-genotype-derived heterogeneity are likely to lie in the enhanced adaptability and hardiness of the population as a whole; promoting the chances that better-adapted phenotypic variants might be able to persist following perturbation and to seed new (heterogeneous) populations [1,2]. Consistent with this, simulations predict that heterogeneous populations can indeed outgrow homogeneous populations during growth in fluctuating conditions [3], and there is a growing body of evidence that such variability is an evolvable trait [4,5]. Consequently, there is currently great interest in unraveling the molecular bases of heterogeneity development.

In microbial populations, phenotypic heterogeneity is sometimes observed as a graded spectrum of gene expression or phenotype [5,6]. In other more striking cases, heterogeneity might be evident as a binary, all-or-nothing, phenotype. This binary phenotype can provide tractable models of biological switch mechanisms. Examples of such all-or-nothing phenomena include expression from the bacterial *lac* and *ara* operons [7,8] and development of competence for transformation in *Bacillus subtilis* [9,10]. It has been predicted that positive auto-regulatory feedback or similar regulatory mechanism can provide a means to underpin the development of these 'bistable' phenotypes amongst cells within a population [11]. Thus, whether or not the concentration of an inducer in a cell surpasses a threshold required for positive feedback and auto-stimulation could, in principle, dictate the phenotypic outcome for many subsequent generations, akin to epigenetic memory. Similar phenomena have been seen in appropriately constructed synthetic regulatory circuits [12,13].

Regulatory requirements for heterogeneity in competence development

Two recently published studies of competence development in *B. subtilis* have helped to crystallize our understanding of the development of bistable expression states. Smits *et al.* [10] dissected out the various regulatory inputs to the natural signal transduction cascade that determines competence development. Similar tools were used by Maamar and Dubnau but with a focus on discriminating between two models of bistability development [9]. A major conclusion derived from both experimental approaches was that the only factor required for the establishment of a bistable pattern in the expression of the *comK* gene, which encodes the master regulator of competence development, is an auto-stimulatory loop of *comK* expression. Thus, among the ~100 genes activated by ComK in cells that are destined to become competent is *comK* itself, but the auto-stimulatory trigger-point is

Corresponding author: Avery, S.V. (Simon.Avery@nottingham.ac.uk).
Available online 19 August 2005