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Some anaerobic protozoa and chytridiomycete fungi possess membrane-bound organelles known as hydrogenosomes. Hydrogenosomes are about 1 micrometre in diameter and are so called because they produce molecular hydrogen[1]. It has been postulated that hydrogenosomes evolved from mitochondria by the concomitant loss of their respiration and organellar genomes[1–4], and so far no hydrogenosome has been found that has a genome[1,2]. Here we provide evidence for the existence of a hydrogenosomal genome of mitochondrial descent, and show that the anaerobic heterotrichous ciliate *Nyctotherus ovalis* possesses a new type of nuclear-encoded 'iron-only' hydrogenase enzyme.

N. ovalis, found in the hindgut of the cockroaches *Periplaneta americana* and *Blaberus* spp.[5], has numerous hydrogenosomes that are intimately associated with endosymbiotic methane-producing Archaea, which use hydrogen produced by the hydrogenosomes (Fig. 1a). The hydrogenosomes are bounded by distinct double membranes and have an inner membrane with cristae-like projections. The matrix contains ribosome-like particles of the same size as the numerous 70S ribosomes of the endosymbiotic methanogenic Archaea (Fig. 1d).

Weak but consistent immunogold labelling was obtained with a commercial antiserum against DNA in more than 80% of the hydrogenosomes we sectioned (Fig. 1b). We labelled the same organelles by using heterologous antisera against an iron-only hydrogenase ([Fe]-hydrogenase[6]) and a hydrogenosomal adenylate kinase of the AK2 type (Fig. 1c). Electron microscopy and immunocytochemistry indicated that DNA, ribosomes and components of a hydrogenosomal metabolism are present in the hydrogenosomes of *N. ovalis*.

Because all hydrogenosomes studied so far lack a genome[1,2], we wondered whether the immunoreactive organelle DNA in *N. ovalis* is functional. By using the polymerase chain reaction (PCR), with primers directed against conserved regions of the mitochondrial small-subunit (SSU) ribosomal RNA genes from ciliates[7,8], we isolated and cloned a fragment of a homologous SSU rRNA gene from *N. ovalis* total DNA. The complete sequence of this rDNA was obtained by rapid amplification of complementary DNA ends, using total RNA from *N. ovalis* as a template. Phylogenetic analysis of conserved regions places the *N. ovalis* sequence with high bootstrap values within the mitochondrial SSU rRNA genes from aerobic ciliates (not shown). Northern blotting reveals that the isolated SSU rRNA gene is abundantly expressed. The cross-hybridizing rRNA is fragmented, supporting the idea of descent from a mitochondrial rRNA gene of a ciliate[7,8] (Fig. 2a).

By using PCR, we identified a nuclear-encoded cDNA 3.6 kilobases long encoding a putative [Fe]-hydrogenase. The cDNA contains a single open reading frame (ORF) consisting of 1,198 codons (Fig. 2e). The amino-terminal half of the predicted polypeptide (residues 22–620) shares 35–41% identity with the [Fe]-hydrogenases from the bacterium *Clostridium* and the proteobacterium *Desulfovibrio*[9]. The middle part of the ORF (residues 630–810) shares 21–24% identity with the HoxE protein of *Synechocystis* spp., the NADP-reducing hydrogenase subunit HndA of *Desulfovibrio fructosovorans*, and the (nuclear-encoded) NuoE/Nuo5 precursors of the 24K (relative molecular mass 24,000) subunit of complex I (NADH-ubiquinone oxidoreductase) from the respiratory chain. The carboxy-terminal part of the ORF (residues 840–1,170) has 28–34% identity with the HoxF genes of *Synechocystis* spp. and *Alcaligenes eutrophus*, and, notably, with the nuclear-encoded NuoF/Nuo6 precursors of the 51K subunit of mitochondrial complex I. In all subunits, the NAD-binding, flavin-mononucleotide-binding and Fe–S motifs are conserved.

The hydrogenase cDNA hybridizes to a 4-kilobase fragment of undigested genomic DNA (Fig. 2d), indicating that the hydrogenase is encoded by gene-sized pieces of *N. ovalis* macronuclear DNA[10] (Fig. 2c). The genomic fragment terminates in a $G_3T_4G_3(T_4G_4)_5$ repeat that is very similar to the telomere sequences of hypotrichous ciliates[10]. The cDNA start was about 180 base pairs downstream from the telomere. The 16 amino-terminal amino acids of the ORF resemble mitochondrial transit peptides. The length of the *N. ovalis* hydrogenase mRNA is in agreement with the sequence data (Fig. 2b).

Our results indicate that *N. ovalis* hydrogenosomes evolved from mitochondria but, contrary to recent predictions[2], they have not relinquished their genome. The evolutionary origin of the chimaeric *N.*

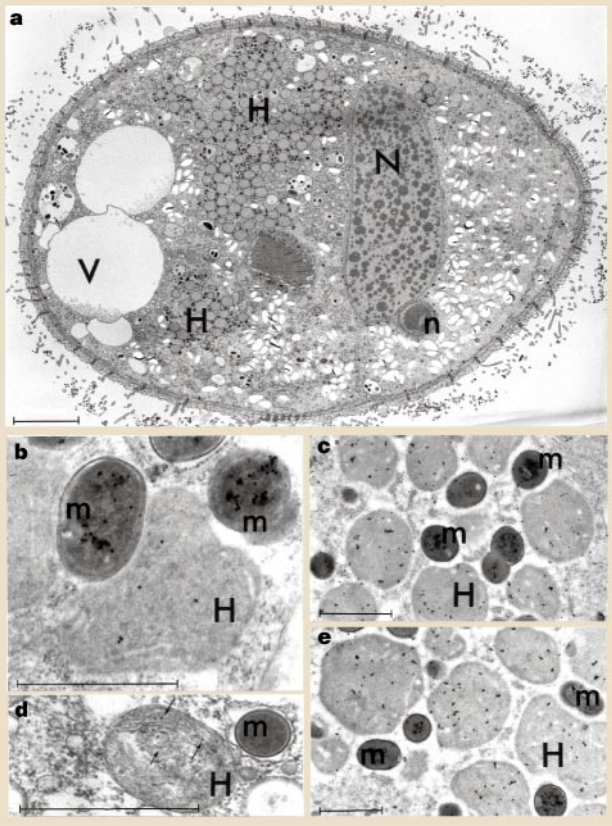

Figure 1 Electron micrographs of *Nyctotherus ovalis* showing hydrogenosomes. **a,** *N. ovalis* from the hindgut of *Blaberus*[5]. The hydrogenosomes (H) are surrounded by endosymbiotic methane-producing Archaea (dark spots); N, macronucleus; n, micronucleus; V, vacuole. Visible by MnO_4 fixation/Epon. **b,c,e,** *N. ovalis* from the hindgut of *Periplaneta americana*[5]. Immunogold labelling of glutaraldehyde-fixed and Unicryl-embedded sections; m, methanogenic Archaea (endosymbionts). **b,** DNA antiserum (Boehringer) labels the matrix of about 80% of the hydrogenosomes on randomly chosen sections with 3–10 grains. The difference in DNA concentration causes the label over the endosymbiotic methanogens to be heavier. **c,** Immunogold labelling obtained with a polyclonal antiserum against hydrogenosomal adenylate kinase (hdgAK2L2) from the anaerobic chytrid *Neocallimastix* sp. L2 (F. V. and B. B., unpublished). Matrix of hydrogenosomes and endosymbiotic methanogens is labelled. **e,** Labelling of the hydrogenosomes (and methanogens) with an antiserum against an [Fe]-hydrogenase of *Trichomonas vaginalis*[6]. There is about 40% amino-acid sequence identity with the [Fe]-hydrogenase described here. **d,** Electron micrograph of a hydrogenosome of *N. ovalis* from *Blaberus*. OsO_4 fixation/Epon. Cristae are clearly visible. Arrows indicate ribosomes. Scale bars are 1 μm, except for that in **a**, which represents 10 μm.

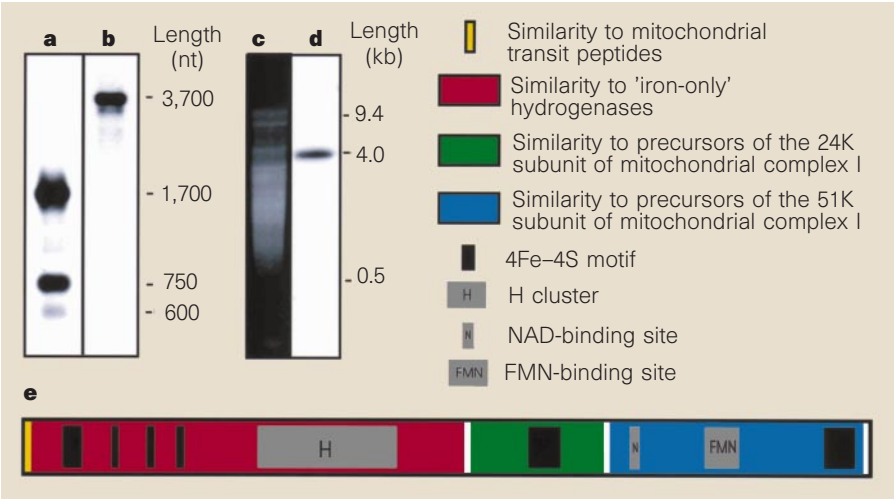

Figure 2 The *N. ovalis* genome. **a,** Northern blot of *N. ovalis* total RNA hybridized to a ^{32}P-labelled fragment of the mitochondrial SSU ribosomal RNA (positions 550–1,660). The largest hybridizing RNA species corresponds in size to the sequenced mitochondrial SSU rRNA, at 1,701 nucleotides (nt). The smaller RNA fragments on the blot probably represent naturally occurring discontinuities, similar to those described for *Paramecium* and *Tetrahymena*[78]. **b,** Northern blot of *N. ovalis* RNA hybridized to the 3' part of the hydrogenase complementary DNA (positions 1,308–3,625). An identical result was obtained when the 5' part of the cDNA was used as a probe. **c,** Ethidium bromide-stained gel with 5 μg undigested genomic *N. ovalis* DNA. Most DNA fragments are gene-sized, at 0.5–10 kilobases (kb). **d,** Southern blot of the same gel hybridized to the ^{32}P-labelled hydrogenase cDNA fragment (positions 1,308–3,625). **e,** The open reading frame encoding the putative [Fe]-hydrogenase. Homologies to known proteins and putative functional domains are indicated. Motifs not to scale.

ovalis hydrogenase gene remains puzzling. Sequence similarity suggests that the hydrogenase couples hydrogen production to the reoxidation of NADH through a combination of functional components derived from respiratory (complex I modules) and fermentative ([Fe]-hydrogenase module) metabolism. The hydrogenase gene could have been inherited from the common proteobacterial ancestor of mitochondria and hydrogenosomes[3]; it may have been acquired through lateral gene transfer from prokaryotes in the *N. ovalis* lineage; or perhaps it was assembled *de novo* in the *N. ovalis* lineage from pre-existing or acquired genetic components.

Hydrogenase expression is clearly a prerequisite for the conversion from mitochondrion to hydrogenosome during the eukaryotic specialization to anaerobic niches. Because hydrogenosomes have arisen independently several times in mitochondrion-bearing lineages[1,4,11], it is possible that their hydrogenases did as well.

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1. Müller, M. *J. Gen. Microbiol.* **139,** 2879–2889 (1993).
2. Palmer, J. D. *Science* **275,** 790–791 (1997).
3. Martin, W. & Müller, M. *Nature* **392,** 37–41(1998).
4. Fenchel, T. & Finlay, B. J. *Ecology and Evolution in Anoxic Worlds* (Oxford Univ. Press, 1995).
5. van Hoek, A. H. A. M., van Alen, T. A., Sprakel, V. S. I., Hackstein, J. H. P. & Vogels, G. D. *Mol. Biol. Evol.* **15,** 1195–1206 (1998).
6. Bui, E. T. N. & Johnson, P. J. *Mol. Biochem. Parasitol.* **76,** 305–310 (1996).
7. Seilhamer, J. J., Olsen, G. J. & Cummings, D. J. *J. Biol. Chem.* **259,** 5167–5172 (1984).
8. Schnare, M. N., Heinonen, T. Y., Young, P. G. & Gray, M. W. *J. Biol. Chem.* **261,** 5187–5193 (1986).
9. Casalot, L. *et al. Anaerobe* **4,** 45–55 (1998).
10. Hoffman, D. C., Anderson, R. C., Dubois, M. L. & Prescott, D. M. *Nucl. Acids Res.* **23,** 1279–1283 (1995).
11. Embley, T. M. *et al. Proc. R. Soc. Lond. B* **262,** 87–93 (1995).

Sequence data have been deposited in the EMBL database under accession numbers Y16775, Y16669 and Y16670.

Did dinosaurs come up to scratch?

The high specificity of bird ectoparasites has frequently been interpreted to mean that they have a long evolutionary history[1]. Either they were present as parasites before the main diversification of birds in the Cretaceous period[2], or they evolved independently into avian parasites on several occasions. The recent discovery of dinosaurs with feathers[3] suggests that birds may have inherited some of their ectoparasites from feathered theropod dinosaurs. We have now found microscopic egg-like structures on the surface of a fossil feather of approximately 120 million years old from the Crato Formation (Lower Cretaceous period) of northeast Brazil. They closely resemble the eggs of ectoparasitic feather mites and indicate an early origin for this specialized group of Acari.

Modern birds have diverse and host-specific ectoparasites[4,5], with many birds being host to several different orders on different parts of the body[6]. The earliest undisputed fossil feathers, of *Archaeopteryx* from the Upper Jurassic period of Bavaria, Germany[7], indicate that ectoparasites could have been using feathers for more than 140 million years. The recent discovery of maniraptoran dinosaurs bearing feathers[3] is suggestive of an even older record, but direct evidence of feather-borne ectoparasites in the Mesozoic era has been lacking.

Here we describe a fossil feather from a famous fossil Lagerstätte, the Nova Olinda Member of the Crato Formation[8], and provide direct evidence that Mesozoic feathers carried parasites. The specimen (National Science Museum of Japan, Tokyo, specimen NSM PV20059) is a slightly asymmetric, probable caudal, plume 85 millimetres in length and with a maximum width of 11 millimetres (Fig. 1a,b).

On the most well-preserved slab there are more than 100 hollow, sub-spherical structures (Fig. 1c), with diameters of 68 to 75 micrometres, adhering to the surface of the rachis and barbs. They resemble the eggs of parasitic Acari[9]. Some spheres are isolated on a single barb, whereas others appear to be concentrated in loose clusters of several spheres. Where examples have split to allow measurement, the walls were between 5 and 7 micrometres thick. They are reddish brown and slightly shiny, which is similar to the preservation style seen in fossil insects from the same deposits[8].

We believe that they are eggs rather than artefacts of preservation because they have a consistent morphology and size; they are restricted to the feather; they are an order of magnitude larger than pyrite framboids, a common spheroidal diagenetic product; and, because of their hollow nature and the circular aperture, 35 to 40 micrometres across (seen on a few of the spheres), are inconsistent with a diagenetic origin. We rule out a spore or pollen affinity on the basis of morphology and the fact that spores and pollens (common in this deposit) are usually organic, rather than preserved as limonitic replacements.

Birds are common hosts to several ectoparasite groups, including fleas, mites and ticks[2]. All three of these arthropod groups lay eggs, but fleas do not usually lay their eggs on the host. Bird lice (order Phthiraptera) cement their eggs to the surface of feathers, with some species being specific to certain parts of the feather, such as the shaft or vane[10,11]. However, the eggs