

Strange fungi with even stranger insides

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Since most mycologists work on ascomycetes or basidiomycetes it is easy to forget chytridiomycetes when thinking about fungi, let alone anaerobic chytridiomycetes. So it was hardly surprising that more than one hundred years ago scientists did not recognise the motile spores in a rumen sample as being fungi. Since it was general knowledge that the rumen was infested with bacteria and ciliates the flagellated cells seen by Liebetanz and Braune (1913; 1910) were considered to be flagellates (see Fig 1). About 50 years later there are some careful remarks in the literature about this *Callimastix* species by Vavra and Joyon (1966) but they do not explicitly mention that it might be a fungus. It took until the seventies before Colin

Orpin finally dared to suggest that those strange anaerobic flagellates that live in the rumen might actually be fungi (Munn *et al.*, 1981; Orpin, 1975; 1977). That scientific dogmas are enormous barriers is obvious from the fact that even the great rumen microbiologist Robert Hungate did not believe that fungi could be anaerobic. The late Rudolf Prins told me that in the sixties, when he and Hungate discovered 'fluffy' colonies in anoxic tubes, they always threw them away because oxygen must have seeped in!

Careful examinations by Heath (1983; 1988b) and Barr (1980; 1988) morphologically linked the anaerobic chytrids to the fungi. Modern phylogenetic analyses have confirmed these findings and reveal the

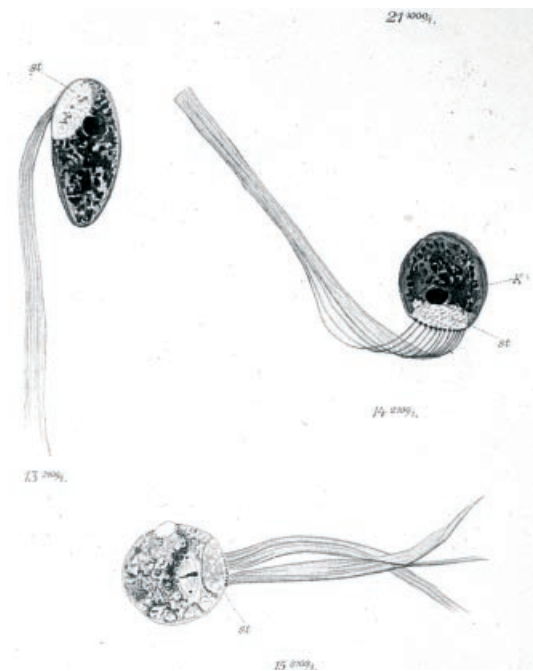
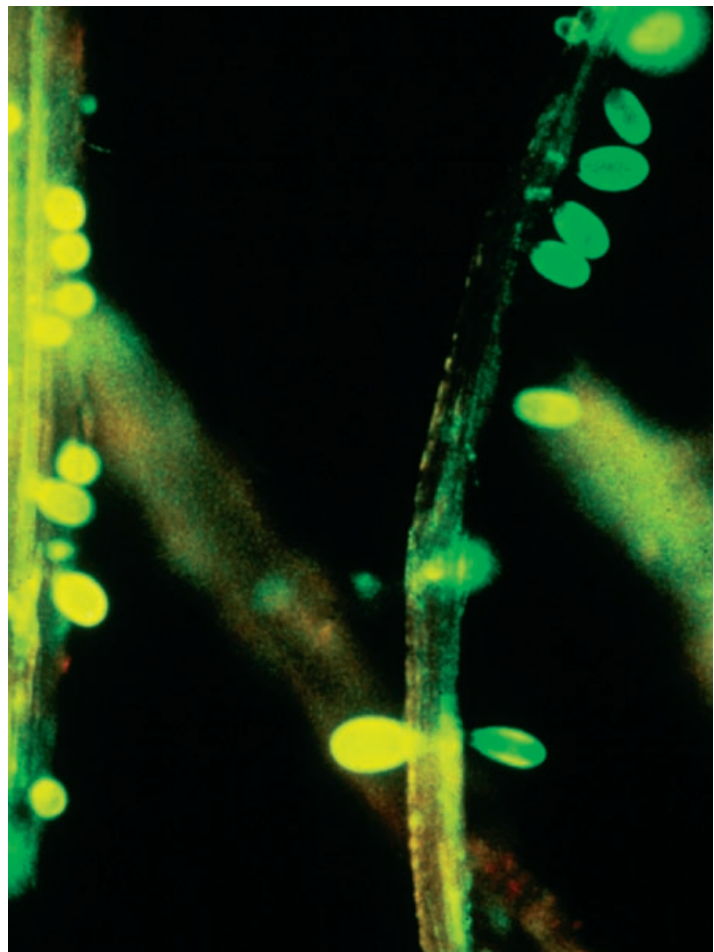


Fig 1 Progress of science. Left: the *Callimastix frontalis* 'flagellates' seen by Robert Braune, taken from a rumen sample in 1913. These are actually the poly-flagellated zoospores of anaerobic fungi. Right: Anaerobic fungi (the vegetative stage, attached to grass leaves) labelled with fluorescent-linked lectin wheat germ agglutinin of *Triticum vulgare*. (photograph kindly provided by Dr. André Breton, Lab. de Microbiologie, INRA, Centre de Recherche de Clermont-Ferrand-Theix, France).



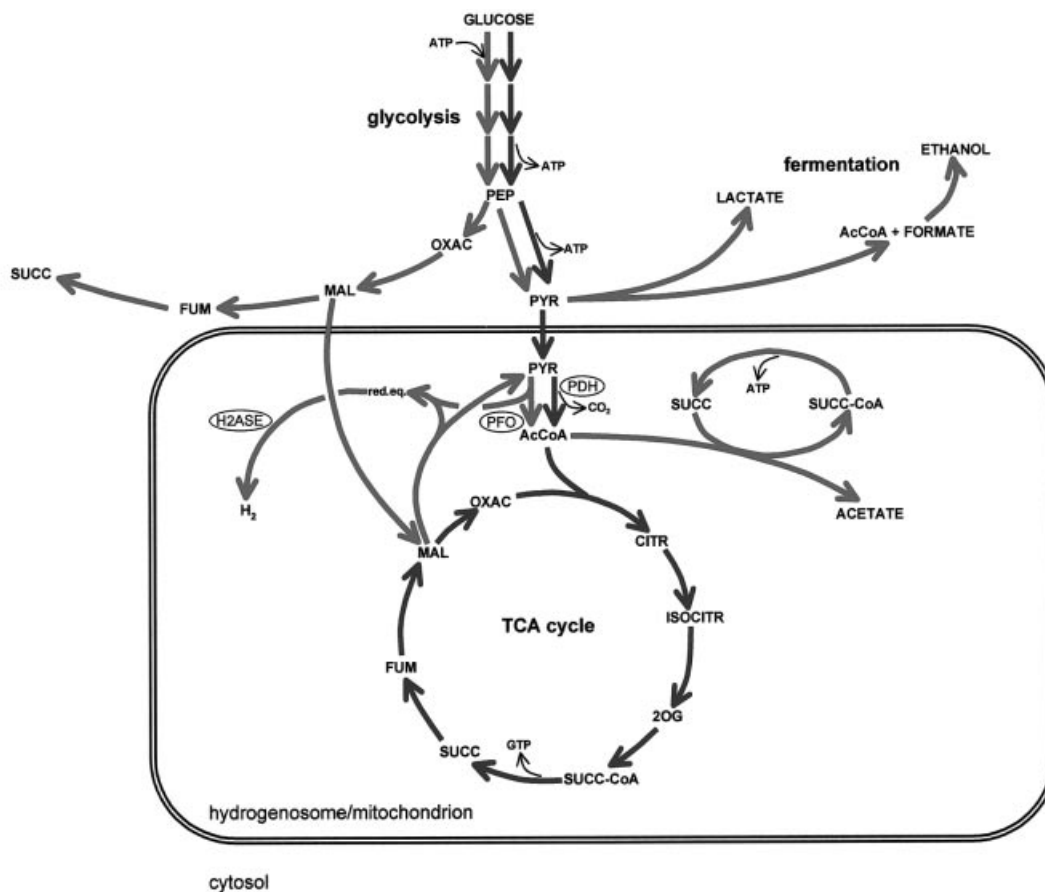


Fig 2 Generalised pathway of aerobic carbohydrate degradation in eukaryotes compared to that of the anaerobic fungus *Neocallimastix frontalis* (Marvin-Sikkema *et al.*, 1993). Aerobic degradation is indicated by blue arrows and the *Neocallimastix* anaerobic route by red arrows. AcCoA, acetyl-Coa; CITR, citrate; FUM, fumarate; H2ASE, hydrogenase; ISOCITR, isocitrate; MAL, malate; 2OG, 2-oxoglutarate; OXAC, oxaloacetate; PEP, phosphoenolpyruvate; PFO, pyruvate: ferredoxin oxidoreductase; PDH, pyruvate dehydrogenase; PYR, pyruvate; red.eq., reducing equivalents; SUCC, succinate; SUCC-CoA, succinyl-CoA. Adapted from van Hellemond *et al.* (1995).

chytrid fungi to be the earliest fungal branch (Bowman *et al.*, 1992; Doré and Stahl, 1991; Li and Heath, 1992; Van der Auwera and De Wachter, 1996).

The narrow ecological niche that these chytrids occupy is the rumen and hindgut of large herbivorous mammals (Trinci *et al.*, 1994). There they assist in the initial breakdown of plant material, as do fungi in most other ecosystems (Heath, 1988a). The interesting thing is that they can do this without any oxygen! Most eukaryotes need oxygen; it is needed to generate large amounts of energy in the mitochondria, the biochemical powerhouses of the cell. When human cells run out of oxygen they start fermenting and make lactate to produce energy, which is obvious from the cramp in the legs after one cannot run any further. So, how do anaerobic fungi cope with the energy-demand under oxygen-free conditions? Interestingly enough, they do not have mitochondria but do have other organelles that produce energy. These organelles are called hydrogenosomes because they make molecular hydrogen. Originally these strange organelles were

thought to be novel 'inventions' to cope with anaerobic conditions (Cavalier-Smith, 1987), but now it has become clear that hydrogenosomes are most likely to be specialised or derived mitochondria (Embley *et al.*, 1997). Hydrogenosomes are not unique to anaerobic fungi, and can be found in a wide variety of anaerobic eukaryotes. Actually, they were first discovered in parasitic trichomonads by Miklós Müller (1973). Most hydrogenosome work has been done on *Trichomonas vaginalis*, the causal agent of a sexually transmitted disease that infects 200 million people annually. Typical mitochondrial features like the electron transport chain, cristae, and more importantly, a genome, are missing from fungal hydrogenosomes. Hydrogenosomes do contain unusual enzymes like hydrogenase and pyruvate: ferredoxin oxidoreductase that replaces the 'normal' mitochondrial pyruvate dehydrogenase. Notwithstanding these peculiar differences, there are sufficient similarities to support the hypothesis that the two organelles share common ancestors. Properties that fungal hydrogenosomes share with mitochondria

include the existence of a transmembrane pH gradient and an alkaline lumen. Free Ca²⁺ pools and calcium phosphate precipitates have also been detected in fungal hydrogenosomes suggesting that, like mitochondria, they accumulate this intracellular messenger (Biagini *et al.*, 1997). Both organelles have a double membrane and use the same import pathway for proteins (see van der Giezen *et al.*, 2002 and references therein). The major function of mitochondria, the conversion of pyruvate to acetyl-CoA (see Fig 2) and ATP production also take place in the hydrogenosomes. Whether other metabolic processes like amino acid metabolism and beta-oxidation also take place in hydrogenosomes is not known at present.

The most important message anaerobic fungi have for us is that it shows there are even more amazing things than the few model organisms which the general textbooks focus on. The fact that anaerobic fungi 'invented' hydrogen-producing mitochondria in order to survive anaerobic conditions demonstrates that cellular organelles which were considered to be defining features of eukaryotes are more plastic than we imagined.

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References

- Barr, D. J. S. (1980) An outline for the reclassification of the Chytridiales, and for a new order, the Spizellomycetales. *Can. J. Bot.* **58**: 2380-2394.
- Barr, D. J. S. (1988) How modern systematics relates to the rumen fungi. *BioSystems* **21**: 351-356.
- Biagini, G. A., van der Giezen, M., Hill, B., Winters, C., and Lloyd, D. (1997) Ca²⁺ accumulation in the hydrogenosomes of *Neocallimastix frontalis* L2: A mitochondrial-like physiological role. *FEMS Microbiol. Lett.* **149**: 227-232.
- Bowman, B. H., Taylor, J. W., Brownlee, A. G., Lee, J., Lu, S., and White, T. J. (1992) Molecular Evolution of the Fungi: Relationship of the Basidiomycetes, Ascomycetes, and Chytridiomycetes. *Mol. Biol. Evol.* **9**: 285-296.
- Braune, R. (1913) Untersuchungen über die im Wiederkauermagen vorkommenden Protozoen. *Arch. Protistenk.* **32**: 111-170.
- Cavalier-Smith, T. (1987) Eukaryotes with no mitochondria. *Nature* **326**: 332-333.
- Doré, J., and Stahl, D. A. (1991) Phylogeny of anaerobic rumen Chytridiomycetes inferred from small subunit ribosomal RNA sequence comparisons. *Can. J. Bot.* **69**: 1964-1971.
- Embley, T. M., Horner, D. S., and Hirt, R. P. (1997) Anaerobic eukaryote evolution: Hydrogenosomes as biochemically modified mitochondria? *Trends Ecol. Evol.* **12**: 437-441.
- Heath, I. B., Bauchop, T., and Skipp, R. A. (1983) Assignment of the rumen anaerobe *Neocallimastix frontalis* to the Spizellomycetales (Chytridiomycetes) on the basis of its polyflagellate zoospore ultrastructure. *Can. J. Bot.* **61**: 295-307.
- Heath, I. B. (1988a) Gut Fungi. *Trends Ecol. Evol.* **3**, no. 7: 167-171.
- Heath, I. B. (1988b) Recommendations for future taxonomic studies of gut fungi. *BioSystems* **21**: 417-418.
- Li, J., and Heath, I. B., (1992) The phylogenetic relationships of the anaerobic chytridiomycetous gut fungi (Neocallimasticaceae) and the Chytridiomycota. I. Cladistic analysis of rRNA sequences. *Can. J. Bot.* **70**: 1738-1746.
- Liebetanz, E. (1910) Die parasitischen Protozoen des Wiederkauermagens. *Arch. Protistenk.* **19**: 19-80.
- Lindmark, D. G., and Müller, M. (1973) Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate *Trichomonas foetus*, and its role in pyruvate metabolism. *J. Biol. Chem.* **248**: 7724-7728.
- Marvin-Sikkema, F. D., Pedro Gomes, T. M., Grivet, J., Gottschal, J. C., and Prins, R. A. (1993) Characterization of hydrogenosomes and their role in glucose metabolism of *Neocallimastix* sp. L2. *Arch. Microbiol.* **160**: 388-396.
- Munn, E. A., Orpin, C. G., and Hall, F. J. (1981) Ultrastructural studies of the free zoospore of the rumen phycomycete *Neocallimastix frontalis*. *J. Gen. Microbiol.* **125**: 311-323.
- Orpin, C. G. (1975) Studies on the rumen flagellate *Neocallimastix frontalis*. *J. Gen. Microbiol.* **91**: 249-262.
- Orpin, C. G. (1977) The occurrence of chitin in the cell walls of the rumen organisms *Neocallimastix frontalis*, *Piromonas communis* and *Sphaeromonas communis*. *J. Gen. Microbiol.* **99**: 215-218.
- Trinci, A. P. J., Davies, D. R., Gull, K., Lawrence, M. I., Bonde Nielsen, B., Rickers, A., and Theodorou, M. K. (1994) Anaerobic fungi in herbivorous animals. *Mycol. Res.* **98**: 129-152.
- Van der Auwera, G., and De Wachter, R. (1996) Large-subunit rRNA sequence of the chytridiomycete *Blastocladiella emersonii*, and implications for the evolution of zoosporic fungi. *J. Mol. Evol.* **43**: 476-483.
- van der Giezen, M., Slotboom, D. J., Horner, D. S., Dyal, P. L., Harding, M., Xue, G.-P., Embley, T. M., and Kunji, E. R. S. (2002) Conserved properties of hydrogenosomal and mitochondrial ADP/ATP carriers: a common origin for both organelles. *EMBO J.* **21**: 572-579.
- van Hellemond, J. J., Klockiewicz, M., Gaasenbeek, C. P., Roos, M. H., and Tielens, A. G. (1995) Rhodoquinone and complex II of the electron transport chain in anaerobically functioning eukaryotes. *J. Biol. Chem.* **270**: 31065-31070.
- Vavra, J., and Joyon, L. (1966) Etude sur la morphologie, le cycle évolutif et la position systématique de *Callimastix cyclopis* Weissensberg 1912. *Protistologica.* **2**: 5-15.