Hydrogenosomes of chytridiomycete fungi: further evidence for a peroxisomal ancestry. FGJ Voncken, JHP Hackstein *(Department of Microbiology and Evolutionary Biology, University of Nijmegen, NL-6525 ED Nijmegen, The Netherlands)*

The acquisition of anaerobiosis in chytridiomycete fungi and the subsequent loss of functional mitochondria, allowed the evolution of a new energy-generating organelle, called the hydrogenosome. Hydrogenosomes of amitochondriate chytridiomycete fungi are chimeric organelles, combining peroxisomal and mitochondrial traits. Apparently, residual nuclear encoded mitochondrial enzymes were forwarded to another pre-existing cell organelle, the peroxisome, in order to facilitate the necessary metabolic functions under anaerobic conditions. Ultrastructural analysis of fungal hydrogenosomes revealed the presence of a single hydrogenosomal membrane and an electron dense matrix, suggesting that these organelles exhibit substantial similarities with microbodies. Consistent with this morphological observation is the presence of a characteristic peroxisomal targeting signal (PTS1), the tripeptide SKL, at the carboxy-terminal end of the hydrogenosomal adenylate kinase. Also the hydrogenosomal malic enzyme carries a putative carboxy-terminal PTS1 signal, the tripeptide KNL. The functionality of both PTS1 signals is shown by expression and immunolocalisation of the native and carboxy-terminal modified hydrogenosomal proteins in the heterologous hosts *Saccharomyces cerevisiae* and *Hansenula polymorpha.* Another peroxisomal characteristic is the presence of a functional β-oxidative pathway in the hydrogenosomes of chytridiomycete fungi. Cellular fractionation, western blotting and immunolocalisation experiments with polyclonal antibodies directed against characteristic peroxisomal marker-enzymes of the β-oxidative pathway, e.g. thiolase and acyl-CoA oxidase, showed accumulation of cross-reacting protein, with the expected M_r, in the hydrogenosomal fraction.

Hsp60 and hsp70 encoding genes in anaerobic chytridiomycete fungi. B Boxma, JHP Hackstein, GD Vogels *(Department of Microbiology and Evolutionary Biology, University of Nijmegen, NL-6525 ED Nijmegen, The Netherlands)*

Anaerobic fungi are inhabitants of the digestive tract of many herbivores, where they play an important role in the digestion of plant material. The anaerobic fungi lack mitochondria, but contain an organelle called the hydrogenosome. The evolutionary origin of the hydrogenosomes is an unsolved question. An endosymbiotic, a cytoplasmic and a mitochondrial origin have been postulated. Ultrastructurally, the hydrogenosomes of anaerobic fungi strongly resemble peroxisomes. Furthermore, the genes encoding the hydrogenosomal adenylate kinase of *Neocallimastix* sp. L2 and *Piromyces* sp. E2 have been isolated. Analysis revealed the presence of a peroxisomal targeting signal, the tripeptide SKL. However, phylogenetic analysis of these genes revealed that they are closely related to orthologous genes encoding mitochondrial adenylate kinases. Therefore, a chimeric origin of the hydrogenosomes is likely (FGJ Voncken, to be published). In order to study whether a mitochondrial import system is still present, we isolated genes encoding