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

for hsp60 and hsp70 of the anaerobic fungi *Neocallimastix* sp. L2 and *Piromyces* sp. E2. Hsp60 and hsp70 are ubiquitous enzymes. In eukaryotes, hsp60 is localised in mitochondria and chloroplasts. Hsp70 is localized in the cytosol, in the endoplasmatic reticulum, in mitochondria and in chloroplasts. Phylogenetic analysis of the hsp60 and hsp70 genes will reveal whether mitochondrial hsp60 and hsp70 genes are recent.

Heterologous production of *Neocallimastix frontalis* xylanases from *Escherichia coli*, filamentous fungi and yeast. R Durand, C Rascle, M Fèvre (*Laboratoire de Biologie Cellulaire Fongique, Centre de Génétique Moléculaire et Cellulaire, CNRS UMR 5534, Bât 405, Université Claude Bernard Lyon I, 69622 Villeurbanne Cedex, France)*

Anaerobic fungi are potent producers of enzymes that degrade the various components of plant cell walls in the rumens of herbivorous animals. The secreted xylanolytic system of *Neocallimastix frontalis* is characterized by a multiplicity of activities, as revealed by zymogram analysis. In order to establish whether these polypeptides are the products of different xylanase genes, or the result of post-translational modifications of the products of a limited number of genes, we started upon the molecular characterization of the xylanolytic system.

Different cDNAs designated *xyn*3 and *xyn*4 were isolated from an expression library of *N. frontalis*, providing evidence for a multigene family in this organism. *Xyn*3 was further characterized and shown to contain a single open reading frame of 1821 bp coding for a protein XYN3 of M_r 66000. The predicted primary structure of XYN3 consisted of two large N-terminal reiterated regions of 223 amino acids showing high homology to each other (88.3%). Each domain of XYN3, XYN3A and XYN3B showed significant homology with fungal and bacterial xylanases belonging to endoxylanase family 11. Analysis of truncated forms of XYN3 confirmed that the full length protein contained two catalytic domains which displayed similar substrate specificity. XYN3A antiserum recognized similar polypeptides in the culture medium of two other rumen fungi *Piromyces rhizinflata* and *Caecomyces communis* indicating that the rumen fungi may secrete similar xylanolytic enzymes. A second cDNA, *xyn*4, that is not homologous to *xyn*3 was shown to encode an endoxylanase consisting of two catalytic domains (A and B) connected by a linker region. The different catalytic domains of *N. frontalis* xylanases were cloned in a bacterial expression plasmid harbouring a 6His-C terminal tag and the recombinant proteins XYN3, XYN3A and XYN4B were purified from *Escherichia coli.* The recombinant proteins hydrolysed xylan to xylo-oligosaccharides.

The availability of recombinant fungal xylanases offers a potentially attractive source of enzymes for industrial applications in the feed, food and paper industries. Chimeric enzymes containing the XYN3A catalytic domain and interdomain linker of the xylanase, fused to one or two XYN4B domains, were produced in *E. coli* cells and purified by affinity chromatography. Experiments are in progress to compare the substrate specificities of the parental domains and of the engineered enzymes.

The coding sequence of *xyn*3A was introduced into different vectors designed