Summary

Biology text books seem to be quite clear about eukaryotic cells. These cells have a nucleus and distinctive organelles, among which the mitochondria are very prominent. Without mitochondria, the cells of animals and fungi would be mostly dependent on the relative inefficient process of glycolysis for their metabolic energy supply.

However, nature has provided much more variety then previously known. Some amitochondriate organisms have developed a unique organelle to cope with anaerobic life. This organelle, known as a hydrogenosome, converts malate or pyruvate to acetate, H_2 and CO_2 with the concomitant production of ATP. Hydrogenosomes were originally discovered in trichomonads but later also found in a variety of other micro-organisms including anaerobic fungi. The organism used in this study was the anaerobic fungus *Neocallimastix frontalis* isolated by my predecessor Femke Marvin Sikkema (1992). My goal was to elucidate the evolutionary origin of the hydrogenosomes of this fungus. Based on a similarity in function, production of ATP by conversion of malate, and the presence of certain enzymes, hydrogenosomes were thought to be biochemically modified mitochondria (Finlay and Fenchel 1989). Another theory, specifically proposed for anaerobic fungi (Cavalier-Smith 1987a), visualized hydrogenosomes as modified peroxisomes. This theory was mainly based upon electron micrographs showing that only a single membrane surrounded fungal hydrogenosomes (Marvin-Sikkema et al. 1992). A careful analysis of the morphology of fungal hydrogenosomes however, revealed two instead of one single membrane (chapter 5). Another indication for a presumed peroxisomal origin of hydrogenosomes was the cross-reactivity of certain hydrogenosomal enzymes with a heterologous polyclonal antibody raised against a peroxisomal targeting signal (Marvin-Sikkema et al. 1993a). When we analysed a cDNA encoding the beta-subunit of the hydrogenosomal homolog of the mitochondrial succinyl-CoA synthetase we could not identify a peroxisomal targeting signal (chapter 2). Actually, there even seemed to be an amino-terminal presequence present on the cDNA with characteristics of mitochondrial targeting signals, such as an enrichment in certain amino acids and an arginine at position -2 relative to the putative cleavage site. A similar presequence was discovered on the cDNA sequence of another hydrogenosomal protein also found in mitochondria, malic enzyme. This presequence is even removed from the mature protein like mitochondrial targeting signals (chapter 3). Targeting experiments in a heterologous host, containing both mitochondria and peroxisomes, supported the suggested relationship to mitochondria (chapter 6). Without the leader sequence the hydrogenosomal malic enzyme was found only in the cytosol of the methylotrophic yeast *Hansenula polymorpha* while the same protein with the leader sequence was targeted to the mitochondria (chapter 6). Additional evidence for a similarity between hydrogenosomes and mitochondria came from the demonstration that hydrogenosomes, just like mitochondria, accumulate cellular calcium (chapter 4).

These and other findings (reviewed in chapter 1) lead to the conclusion that hydrogenosomes in anaerobic fungi are most likely modified mitochondria.