

1 The Road to Hydrogenosomes

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1.1 Introduction

Recent studies increasingly support the hypothesis that organelles originating from the mitochondrial endosymbiotic event comprise a family of diverse structures and not only the typical mitochondria, with their characteristic cristate structure functioning as aerobic powerhouses of cells. This large family is regarded today to encompass a broad, almost continuous spectrum of organelles, from the much-studied typical aerobic mitochondria, through anaerobic mitochondria and hydrogenosomes to the still-enigmatic mitosomes. It is becoming generally accepted that these organelles evolved from a common ancestral organelle that arose in a single endosymbiotic event sometime during the emergence of the eukaryotic cell, as we know it today. Contributions to this volume explore the diverse members of this organelle family in detail and most of them also give a glimpse of the intense debates on the evolutionary origins of these organelles and their current impact on general hypotheses on the origin and evolutionary diversification of eukaryotes.

One of the key contributions leading to the prevalent current views was the discovery of hydrogenosomes in certain “anaerobic” flagellates in the early 1970s. Initially they were regarded as an oddity or aberration and it took time before they were seriously discussed outside the community of protistologists. They remained an enigma until their mitochondrial relationships received strong support in the 1990s. Recent developments overshadow the early days of hydrogenosomes, as they indeed should. This volume, presenting an exciting collection of papers on mitochondrial diversity and evolution, is perhaps the appropriate place to retell briefly those early days by someone who was there. I eagerly accepted the suggestion of my friend, the coeditor of this volume, Bill Martin, that I do this. Needless to say, this will be an intensely personal history and certainly not an autobiography. Since the contributions to this volume provide a clear picture of later developments, I restrict my story to the very early days, approximately the first 12 years after the discovery of hydrogenosomes (Lindmark and Müller 1973).

1.2 The Story

I start in *medias res*. After having spent some years in Budapest exploring protist food vacuoles and lysosomes with histochemical methods, I was given the opportunity of my life by Christian de Duve, who received the Nobel Prize in 1974 for the discovery of lysosomes and peroxisomes. In 1964 he invited me to join his laboratory at the Rockefeller University (still called Institute then) to explore these organelles with biochemical and cell fractionation methods in protists. My work in New York started well, although I was a novice to biochemistry at that time. Soon I was able to characterize biochemically the lysosomes of the ciliate *Tetrahymena pyriformis* and also demonstrated classic peroxisomes in this species (Baudhuin et al. 1965). This finding showed the peroxisome to be broadly distributed in eukaryotes, since until then they had only been detected in mammalian tissues. After a year in New York, I spent a year at the Carlsberg Laboratories in Copenhagen under the guidance of Heinz Holter and Cicily Chapman-Andresen working on lysosomes and peroxisomes of amoebae. I returned to de Duve's laboratory in 1966 and continued working on *T. pyriformis*. With James Hogg of Queens College, we demonstrated that *T. pyriformis* peroxisomes unexpectedly contain two enzymes of the glyoxylate bypass of the glyoxylate cycle responsible for gluconeogenesis from acetyl-coenzyme A. The rest of the cycle in this ciliate was localized in the mitochondria, an interesting intracellular division of labor (Müller et al. 1968). About the same time, certain plant peroxisomes were found by others to harbor the glyoxylate cycle and were named glyoxysomes. In the laboratory we became interested in the distribution of peroxisomes in the living world, looking for new organisms to explore. Little did I suspect that this quest would lead to a "novel" organelle, the hydrogenosome. I have recounted these events in some detail in a Past President's address given to the Society of Protozoology (Müller 1985).

The theory of endosymbiotic origin of chloroplasts and mitochondria was in the air in the late 1960s. Lynn Margulis had just published her first paper on the serial endosymbiotic theory. As part of our studies on lysosomes and peroxisomes, the question of the evolutionary origin of these other organelles was also often discussed. The lysosomes could be relatively easily derived from the cell membrane, a conclusion that is still valid today. The origin of the peroxisomes, single membrane-bounded organelles that contain direct oxidases and catalase, was a more complicated question. de Duve (1969) suggested a modified serial endosymbiotic hypothesis (Fig. 1.1). The events envisaged by him invoked as a host cell an ancestral anaerobic phagocytic eukaryote without any respiratory organelles. In a first endosymbiotic event it was supposed to take up and integrate an aerobic bacterium containing direct oxidases and no cytochrome-mediated electron transport chain. This became the peroxisome. The uptake of a bacterium with a cytochrome-mediated respiratory system in a subsequent, second

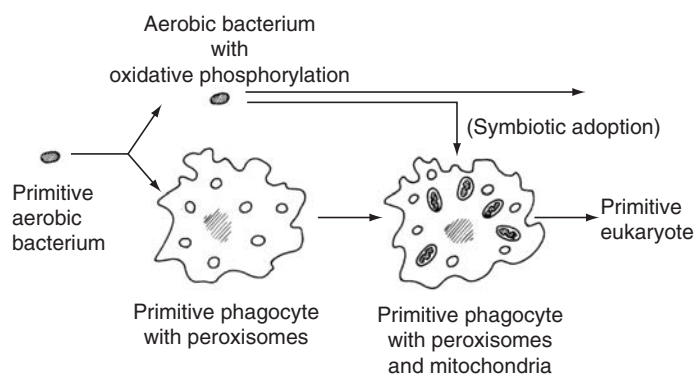


Fig. 1.1. Schematic representation illustrating de Duve's hypothesis concerning the evolutionary origin of peroxisome. (Figure 3 in de Duve 1969)

endosymbiotic event lead to the formation of a protomitochondrion, resulting in a complex eukaryotic cell with two respiratory organelles, the peroxisome and the mitochondrion.

An important corollary of this hypothesis was the possible existence of organisms that derived from an intermediary stage already containing peroxisomes but not yet mitochondria. The search for extant representatives of such organisms was an interesting challenge. One major motivation in trying to find these intermediates in eukaryogenesis was the hope that they could provide insight into the ancestral function of peroxisomes, which in the late 1960s were already known to vary significantly in their enzymatic composition from organism to organism. As proposed by de Duve:

"In a more speculative vein, our considerations of the role peroxisomes may have played in a primitive cell devoid of mitochondria could be relevant to the physiology of microorganisms that either do not have mitochondria ... or have deficient mitochondria. ... Perhaps a survey of microorganisms, inspired by these considerations, may disclose the existence of eukaryotic organisms truly devoid of mitochondria, but possessing peroxisomes, in which the postulated ancestral function of these particles could be explored and assessed." (p. 380 in de Duve 1969)

While I continued my biochemical studies on *T. pyriformis*, I eagerly began a search for such protists, too.

Trichomonad flagellates seemed to be a good first choice. The human pathogen *Trichomonas vaginalis* and the cattle pathogen *Tritrichomonas foetus* were much-studied species available in bacterium-free cultures and were thus amenable to biochemical and cell fractionation studies, approaches extensively practiced by our group. The available physiological data showed that the respiration of these species was not of mitochondrial type, because it could not be inhibited with cyanide and other mitochondrial inhibitors. Furthermore no cytochromes were detected in these trichomonads (Ryley 1955).

A biochemical peculiarity of the trichomonads was their essentially anaerobic nature, their independence from the availability of molecular oxygen as a terminal electron acceptor, a point that soon gained much importance. At the same time, the ultrastructural data accumulated revealed the absence of typical cristate mitochondria. Instead the well-known paracostal and paraxostylar granules of mitochondrial size turned out to resemble microbodies, by that time known to correspond to peroxisomes in many aerobic organisms.

We attempted to test the hypothesis that the granules seen in trichomonads are related to peroxisomes. The presence of cyanide-insensitive respiration and their characteristic morphology seemed to point in this direction. In addition, one of the two species, *T. foetus*, was known to contain catalase, the marker enzyme of peroxisomes. In 1971 I obtained cultures of this organism, verified the presence of catalase activity and performed a seemingly obvious experiment. I prepared a homogenate in isosmotic sucrose, and separated it by centrifugation into sediment and supernatant, with the expectation that the sediment containing the granules would contain most of the catalase activity. The results were disappointing: most of the activity was recovered in the supernatant. The total recovery of the activity was close to 100%, indicating no problems with the fractionation experiment itself. Two repeat experiments gave the same results. Since I well “knew” that catalase had to be in the organelles, I assumed that they had been broken during homogenization owing to their excessive fragility and decided that this approach would not lead me to the desired goal. I turned to other organisms with cyanide-insensitive respiration, work that did not lead to the desired breakthrough.

This disappointment did not leave me in peace for long, however. Reflecting on the data, I finally was willing to accept them at face value. OK, the organelles were not the subcellular location of catalase! But then what were they? A dominant structure occupying a significant portion of the cell volume, as readily seen in the electron microscope, surely has some important role to play. Since this was the only large, membrane-bounded entity seen in trichomonads, its relationship to either mitochondria or peroxisomes was still a possibility. The alternative assumption of having an independent origin remained open of course. But the immediate task was to define the *biochemical* nature of the organelle, the possible relationships to either mitochondria or peroxisomes only being important in suggesting enzymes and functions to explore.

Returning to the problem, I looked for typical mitochondrial or peroxisomal enzymes in *T. foetus* cell homogenates and fractions obtained by different centrifugation methods, but all results were negative (Müller 1973). It occurred to me then that this enigmatic organelle might be somehow connected to the anaerobic nature of the organisms already mentioned. A tempting possibility was that they could be somehow involved in the production of hydrogen by these organisms, a metabolic end product rarely found among eukaryotic organisms. Donald G. Lindmark, whose thesis work was on

hydrogen production in anaerobic prokaryotes, joined our group and we set out to test our assumption.

We assayed homogenates of *T. foetus* for enzymes known to be involved in hydrogen formation in anaerobic bacteria and we detected the presence of pyruvate:ferredoxin oxidoreductase and hydrogenase, enzymes fundamentally different from the pyruvate-metabolizing enzymes of mitochondria. This confirmed the results of Bauchop (1971), who demonstrated that hydrogen production by *T. vaginalis* is biochemically analogous to processes in *Clostridium* species. Assays of subcellular fractions obtained by diverse centrifugation methods localized these activities to a large granule fraction that was shown by electron microscopy to be significantly enriched in granules, identifiable with the paracostal and paraxostylar granules. These results strongly suggested that the granules are indeed the subcellular site of hydrogen production. We published our first paper on these results in the *Journal of Biological Chemistry* in 1973, where we concluded:

“These findings underscore the unique nature of the microbody-like particles of *T. foetus*. In contrast to mitochondria or peroxisomes, in which electron transfer is directed toward molecular oxygen, they utilize protons as terminal electron acceptors and thus produce molecular hydrogen. We propose the term “hydrogenosome” to designate this new biochemically defined subcellular entity.” (p. 7728 in Lindmark and Müller 1973)

I wish to stress the point that our definition was based on the biochemical properties of the organelle and did not extend to its morphological characteristics, leaving open the possibility that hydrogenosomes, if and when detected in other organisms, could display a morphology different from that observed in trichomonads.

Our immediate goals were twofold: first, to see whether hydrogenosomes are a peculiarity of *T. foetus* or are present also in other trichomonads as well as in other protist groups; second, to characterize the biochemistry of the newly discovered organelle in more detail. Along the first line of inquiry, we studied two additional trichomonad species with the same methods. The results were identical for the important human parasite *T. vaginalis* (Lindmark et al. 1975) and for *Monocercomonas* sp. (Lindmark and Müller 1974a), a species regarded as a primitive form at that time. The selection of these two species reflected our interest in the possible medical and evolutionary significance of the new organelle. The publication of our *T. vaginalis* results met unexpected and amusing obstacles, however. We submitted our detailed manuscript to the *Journal of Parasitology*, regarding it as the most appropriate venue to reach applied and general parasitologists alike. The paper was *not* accepted for publication because the reviewer stated that its content is self-evident and does not contribute any new information. This was at a time when practically no parasitologist or biologist was even aware of the existence of hydrogenosomes. Finally we summarized our data in a brief communication (Lindmark et al. 1975). We felt that the case for hydrogenosomes as a unique type of organelle in trichomonad flagellates had

Origin of Mitochondria and Hydrogenosomes

Martin, W.F.; Muller, M. (Eds.)

2007, XVIII, 306 p. 38 illus., 7 illus. in color., Hardcover

ISBN: 978-3-540-38501-1