

Were mitochondrial contractions driving the cellular energy cycle?

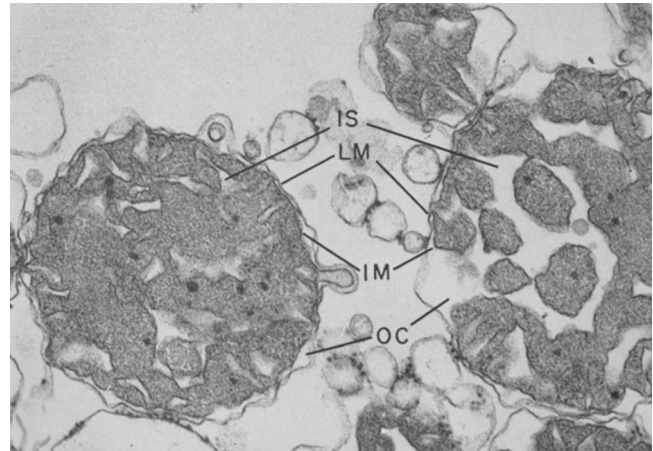
No, but Charles Hackenbrock's thesis work so elegantly supported the hypothesis—that a mechanochemical mechanism coupled electron transport to ATP synthesis—that it was cited almost 600 times as evidence. It earned him speaking invitations all over the US and Europe, and an assistant professorship at Johns Hopkins University (Baltimore, MD) straight out of graduate studies. "Everyone," he says, "fell in love with these ultrastructural changes."

Hackenbrock, now an emeritus professor at the University of North Carolina, Chapel Hill, recalls that the project started as a graduate course project to isolate mitochondria from rat livers and test the effects of snake venom on their function. But when he noticed that his control group of mitochondria underwent a dramatic conformational change—from "knotted up" just after isolation to the "beautiful mitochondria" of intact cells—during a sucrose buffer incubation, he immediately switched his thesis to study how this structural change might correlate with oxidative phosphorylation function.

"In those days, mitochondrial function in terms of making ATP was one of the key questions in biochemistry around the world," he says. "I realized immediately this was going to be of wide interest." At the time, the only evidence for structural changes during oxidative phosphorylation came from light-scattering studies that measured the optical density of isolated mitochondria (Chance and Packer, 1958). Lehninger (1959) first proposed that the structural changes might represent, in Hackenbrock's words, "an energy-linked mechanochemical process which may reside in a multienzyme respiratory assembly which carries out electron transport and oxidative phosphorylation." In one scenario for such a link, ions might be pumped across the inner membrane to generate osmotic deformation of the mitochondrion, and the resultant mechanical force might then be used by an enzyme to generate ATP.

Hackenbrock developed a unique set-up to correlate ultrastructural changes, light scattering, and metabolic function. Before and after addition of ADP and substrates for the electron transport chain, he measured mitochondrial optical density and oxygen consumption and fixed mitochondria for EM (Hackenbrock, 1966).

The experiment revealed that mitochondria changed from a "condensed" to "orthodox" conformation while incubating in a buffer supporting slow respiration with no added ADP. Once ADP was added, however, the organelles contracted to the condensed form once again. The contractions were reversible (arguing against a fixation artifact) and seemed to be controlled by the inner mitochondrial membrane, which shrank away from the outer membrane and enclosed a more dense matrix in the condensed form. In a follow-up study, Hackenbrock clearly linked the contraction phenomenon to the activity of the electron transport chain by using several electron transport inhibitors and then adding downstream substrates to reinitiate transport and conformational changes (Hackenbrock, 1968).



Transitions between orthodox (left) and condensed (right) forms of mitochondria were suggested as a driving force for ATP generation.

But the story that unfolded would eventually support the concurrent and competing chemiosmotic hypothesis proposed by Mitchell (1961). Peter Mitchell suggested that there was not a direct, mechanical linkage in coupling, but instead an indirect build up of a proton gradient across the inner mitochondrial membrane, with the potential energy of protons moving back across the membrane somehow driving ATP synthesis.

The discovery that the electron transport enzymes were, in fact, acting as proton pumps (Mitchell and Moyle, 1965) and that the ATP synthetase molecule could transform the potential energy of the protonmotive force into mechanical energy to bring ADP and phosphate together (Boyer, 1975) clinched the chemiosmotic coupling theory. Mitchell and Boyer won Nobel Prizes in Chemistry in 1978 and 1997, respectively, for their work.

As Mitchell's work was unfolding, Hackenbrock was not the only one working on alternative theories. By the mid-1960s, according to Mitchell's Nobel Lecture, "the field of oxidative phosphorylation was littered with the smouldering conceptual remains of numerous exploded energy-rich chemical intermediates." Hackenbrock, at least, was on the right track in terms of looking for a structural rather than chemical mediator of energy transformation. The true movement was in the conformation of a protein, not of a whole membrane, but Hackenbrock says his work was "on a continuum of emphasis on some kind of conformational movement." Meanwhile, some in the field continue to believe that gross membrane movements might fine-tune metabolism rates, perhaps by affecting the formation of electron transport chain supercomplexes. But whether this is relevant in cells in animals, which have very stable ATP levels, is still up for grabs. **JCB**

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