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Discussion

Historical introduction

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A number of indirect observations in the 1950s had suggested that mitochondria could accumulate Ca^{2+} (see [1] for an historical review), but direct proof of the suggestion was only provided in 1961–1962 by Engstrom and De Luca and Vasington and Murphy [2,3]: they showed that isolated mitochondria did actually accumulate large amounts of Ca^{2+} in a process that was energized by the activity of the respiratory chain or by the hydrolysis of added ATP. The discovery triggered a large number of studies that rapidly established a number of the properties of the process. It was thus shown that the uptake of Ca^{2+} was an alternative to the synthesis of ATP in the usage of respiratory energy, the same amount of energy been required to phosphorylate one ADP molecule or to transfer two Ca^{2+} ions into mitochondria. It was also found [4] that phosphate anion accompanied Ca^{2+} in its transfer across the mitochondrial membrane to precipitate it in the matrix as an insoluble salt. Remarkably, even before the actual uptake of Ca^{2+} had been demonstrated [2,3], Saris had found that the addition of pulses of Ca^{2+} to mitochondria elicited the ejection of H^+ [5]. It was an important observation, as it placed the mechanism of the Ca^{2+} uptake process, which had been generally interpreted along the orthodox lines of the then domineering "chemical coupling" mechanism [6], within the framework of the chemiosmotic concept [7] which in those early days was moving its first hesitant steps. The Ca^{2+} uptake reaction had the canonical properties of carrier-mediated membrane transport processes, i.e., it exhibited saturation kinetics, was competitively inhibited by Sr^{2+}, and was specifically abolished by very low concentrations of the inhibitor ruthenium red: the idea of a Ca^{2+} carrier in the inner mitochondrial membrane thus became a generally accepted proposal.

For a number of years after its discovery, the process of Ca^{2+} uptake by mitochondria was one of the most popular topics in bioenergetics, and its entire phenomenology was rapidly characterized by findings in a number of Laboratories. One of these findings, however, was disturbing: the affinity of the uptake system for Ca^{2+} was very low: measurements on isolated mitochondria with different methods and in several Laboratories invariably yielded apparent K_m values of the order of 5 μM or higher, which were further increased by Mg^{2+} added to mitochondria in the concentrations known to exist in the cytosol (see [8] for an early review). The finding was disturbing because at that time the main reason for the popularity of the Ca^{2+} uptake process was its possible role in the regulation of cell Ca^{2+}. Since the concentration of Ca^{2+} in the cytosol was known to be in the sub-μM range, the poor Ca^{2+} affinity of the uptake system was hard to reconcile with such a role. The idea thus gained momentum that mitochondria would not be able to significantly handle Ca^{2+} in the intracellular ambient. The matter, however, was not so simple. Mitochondrial uptake of Ca^{2+} had somehow to occur in vivo, its poor affinity for Ca^{2+} notwithstanding. This was demanded by findings made by Denton and McCormack on the regulation of the TCA cycle in the mitochondrial matrix, and thus on the eventual delivery of reducing equivalents to the respiratory chain to synthesize ATP (reviewed in [9]). Two dehydrogenases of the cycle, and the phosphatase that dephosphorylates pyruvic acid dehydrogenase phosphate were found to be exquisitely sensitive to Ca^{2+}, which thus had to be somehow transported to the mitochondria matrix in a regulated fashion. Then, our Laboratory had found that more than 50% of a pulse of radiocalcium injected into rats was recovered in the mitochondrial fraction of liver homogenates (later on of other tissues as well), implying that Ca^{2+} uptake had occurred in mitochondria *in vivo* [10]. Importantly, this percentage dropped to less than 20% if the rats had been injected with an uncoupler of mitochondrial energy transformation prior to the injection with radiocalcium, showing that the uptake of Ca^{2+} in the liver had evidently occurred via the energy-linked process that had been characterized on isolated mitochondria. A conundrum, clearly: which remained unsolved for decades. The way out of it was only found in the 1990s by Pozzan, Rizzuto and their coworkers, who showed that the bulk concentration of Ca^{2+} in the cytosol does not reflect its concentration in the immediate vicinity of mitochondria, where it can actually reach levels well in the μM range [11,12]. This is so because micropools of Ca^{2+} concentration high enough to satisfy the poor affinity of the uptake system are generated around mitochondria by the release of large amounts of Ca^{2+} from vicinal endoplasmic reticulum.

The search for the putative carrier of Ca^{2+} (defined as a Ca^{2+} uniporter in chemiosmotic parlance) had started early using a number of conventional experimental protocols. In the course of decades it had produced numerous uniporter candidates, none of which, however, survived the necessary conclusive tests (reviewed in [13]). The renewed interest in the process of mitochondrial Ca^{2+} uptake triggered by the Ca^{2+} micropools concept then brought fresh impetus to the search for the elusive uniporter. Genoming profiling approaches which were not available in the early days, coupled to modern molecular biology technologies eventually led to its molecular identification. One critical tool, here, was a finding

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we had made long ago: yeast mitochondria failed to take up Ca^{2+}, i.e., they evidently did not possess the putative Ca^{2+} carrier [14].

As will be discussed in the following papers by the Authors who have been responsible for the recent spectacular advances, their recent discoveries have revealed that the Ca^{2+} uptake system has a very high degree of molecular and functional complexity. That the system could be represented by more than one component was per se not unexpected, as the concept of a peripheral, Ca^{2+} recognizing component that would somehow deliver Ca^{2+} to the membrane-intrinsic transporter had been already aired in the past [15]. What was unexpected, however, was the amazing degree of complexity of the system. Which will fuel discussions for a long time to come.

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