The Selfish Mitochondrion

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Mitochondria match ATP supply to fluctuations in cellular energy requirements. Cellular energy requirements are often signaled to mitochondria by increases in the cytoplasmic concentration of Ca^{2+} ions. These increases in Ca^{2+} reach the mitochondrial matrix to regulate the proteins, enzymes and transporters required for mitochondrial ATP synthesis. Mitochondria may also limit energy demands by capturing the ion and shaping cytosolic Ca^{2+} signaling. Mitochondrial uptake of Ca^{2+} is driven by the substantial electrochemical gradient generated by the respiratory chain complexes. Although functional studies since the 1960s hinted at the nature of the Ca^{2+} channel involved, the molecular identity eluded physiologists until 2011 when the mitochondrial Ca^{2+} uniporter (MCU) gene was identified[1,2]. MCU is a Ca^{2+}-selective inwardly rectifying channel and a major pathway for the passage of Ca^{2+} ions from the cytoplasm into mitochondria. However, while well understood at the genetic and protein level, how MCU regulates cell function is unresolved. Understanding MCU regulation is of the utmost importance as disturbances in mitochondrial Ca^{2+} uptake accompanies several human diseases[3].

Mitochondrial regulation of the Ca^{2+} store

Mitochondrial Ca^{2+} uptake via MCU regulates cell function by controlling cytoplasmic Ca^{2+} concentration. The uptake facility allows mitochondria to control IP_3-mediated Ca^{2+} release by regulating store-operated Ca^{2+} entry (SOCE; a key mechanism that replenishes internal Ca^{2+} stores), Ca^{2+} transfer to the internal store, and by modulating the channels responsible for Ca^{2+} release[4,5]. In each case, mitochondria's control of IP_3-mediated Ca^{2+} signaling generates parallel changes in the cytoplasmic Ca^{2+} concentration.

Yoast *et al.* have now reported a disconnection between mitochondrial control of the Ca^{2+} increase arising from SOCE, and the current (I_{CRAC}) that gives rise to the Ca^{2+} increase[6]. Using an array of functional protocols, the authors' found that CRISPR/Cas9 MCU knockout decreased SOCE in a range of cells, while paradoxically increasing the Ca^{2+} rise from SOCE. The experiments reveal that MCU activity increases the overall current and local Ca^{2+} rise occurring via I_{CRAC}, but decreases the global Ca^{2+} rise occurring via SOCE. The major effect of MCU on cytoplasmic Ca^{2+} appears to arise from the organelle's accumulation of the ion and not from the control of Ca^{2+} influx; mitochondria hoard so much Ca^{2+} that they limit rather than promote store refilling. Store refilling occurs, but only after mitochondria have acquired their "share" of Ca^{2+}.

A comparison of electrophysiological properties revealed that a slow Ca^{2+}-dependent inactivation of I_{CRAC} was accelerated by MCU knockout, and reduced by 'energized' mitochondria that presumably buffer cytoplasmic Ca^{2+} more effectively[6]. The prevailing proposal for the effects of MCU on I_{CRAC} hinges on a negative feedback loop in which a rise in cytoplasmic Ca^{2+} inactivates the channels. By buffering incoming Ca^{2+}, mitochondria diminish

this feedback to prolong Ca^{2+} influx[7–9]. That MCU knockout increases the rise in cytoplasmic Ca^{2+} caused by SOCE, while SOCE is reduced, is counterintuitive to this model. To explain this, the authors put forth the simple idea that mitochondrial accumulation of the ion reduces cytoplasmic Ca^{2+} and limits store refilling. Given that Ca^{2+} stores refill faster and store content is increased when MCU is disabled, mitochondrial Ca^{2+} homeostasis appears to take priority over the organelles control of the internal store content.

MCU knock-out also increased the frequency of agonist-induced cytosolic Ca^{2+} oscillations, NFAT translocation, and B-lymphocyte proliferation. These effects likely arise from an increased cytoplasmic Ca^{2+} concentration occurring in stimulated MCU-deficient cells. However, the authors[6] used optical patch clamp Ca^{2+} imaging to show that MCU knockout has no effect on elementary IP_3 receptor (IP_3R) activity. These results appear at odds with the known role of mitochondria in regulating IP_3-evoked Ca^{2+} release, by buffering cytoplasmic Ca^{2+}, and modulating Ca^{2+} store content (hence the driving force for Ca^{2+} release), and raise an important question. Why is IP_3R activity unaltered in MCU knockout when the store content is increased? Perhaps IP_3R activity is decreased to precisely offset the increased Ca^{2+} store content. This seems unlikely, but a lack of other plausible alternatives makes it clear that there is much still to be resolved to fully understand MCU regulation of cell physiology

Future work to unravel mitochondrial Ca^{2+} control complexity

A quantitative analysis of store Ca^{2+} concentrations and Ca^{2+} oscillation parameters will directly address how IP_3-mediated Ca^{2+} release alone is unaltered in the absence of uniporter activity. But contemporary genome editing effects also contrast with those obtained using siRNA knockdown[10] and highlight cell-type specificity in certain features of MCU function. For example, in HEK293 cells, mitochondrial Ca^{2+} uptake does not alter mitochondrial Ca^{2+}, SOCE, or IP_3R activity, but does modulate Ca^{2+} release. What other cell/tissue-specific roles does MCU play? In this regard, pharmacological mitochondrial uncoupling accelerates Ca^{2+} decline from I_{CRAC} inactivation in HEK293 cells lacking MCU, but not RBL-1 or Jurkat cells. The authors suggest accelerated decline may be related to free radical generation. However, given that severe mitochondrial uncoupling can deplete cytosolic ATP, and ATP levels, the ATP/ADP ratio, or protein kinases may offset MCU effects on I_{CRAC}, the effect of mitochondrial uncoupling is difficult to interpret.

In raising these questions, Yoast *et al.*[6] , have used the power of modern molecular physiology to provide new insight into MCU regulation. They demonstrate that mitochondrial Ca^{2+} uptake is complex and that additional efforts are required to complete our understanding of the multifaceted contributions of MCU to the physiological control of Ca^{2+} signaling. This is of the utmost importance for our understanding of pathophysiology in which dysfunctional mitochondrial Ca^{2+} signaling is involved[3].

References

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