The gatekeepers of mitochondrial calcium influx: MICU1 and MICU2

Malini Ahuja & Shmuel Muallem

The receptor-evoked Ca^{2+} signal is sensed and translated by mitochondria. Physiological cytoplasmic Ca^{2+} ($[\text{Ca}^{2+}]_c$) oscillations result in mitochondrial Ca^{2+} ($[\text{Ca}^{2+}]_m$) oscillations, while large and sustained $[\text{Ca}^{2+}]_c$ increase results in a pathological increase in basal $[\text{Ca}^{2+}]_m$ and in Ca^{2+} accumulation. The physiological $[\text{Ca}^{2+}]_m$ signal regulates $[\text{Ca}^{2+}]_c$ and stimulates oxidative metabolism, while excess Ca^{2+} accumulation causes cell stress leading to cell death. $[\text{Ca}^{2+}]_m$ is determined by Ca^{2+} uptake mediated by the mitochondria Ca^{2+} uniporter (MCU) channel and by Na^+ - and H^+ -coupled Ca^{2+} extrusion [1].

See also: K Kamer *et al* (March 2014)

Recent developments led to the elucidation of the molecular components of the mitochondrial Ca^{2+} homeostatic machinery. Mitochondrial Ca^{2+} extrusion is mediated by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger isoform NCLX. The characterization of the uniporter complex began with the finding of the mitochondrial Ca^{2+} uptake 1 (MICU1). This was followed by finding of the pore forming mitochondrial Ca^{2+} uniporter (MCU) (reviewed in [1]), and the MCU paralog MCUB, which does not conduct Ca^{2+} but inhibits mitochondria Ca^{2+} influx when present in the MCU complex [2]. Most recently identified were MICU2 [3] and the essential MCU regulator (EMRE), which mediates interaction of MICU1 and MICU2 with MCU [4]. The tight regulation of mitochondrial Ca^{2+} influx and highly cooperative dependence of mitochondrial Ca^{2+} influx on $[\text{Ca}^{2+}]_c$ require control of MCU channel function, which is in turn mediated by MICU1

and MICU2. The function of MICU1 is the subject of intense investigation [5–7]. In this issue of *EMBO reports*, Kamer and Mootha describe the function of MICU2 and its relation to MICU1 and MCU [8].

MICU1 and MICU2 have two Ca^{2+} -binding EF hands and function as the MCU Ca^{2+} sensors. Two studies examined the function of MICU1 in detail and concluded that MICU1 functions as a gatekeeper that sets the Ca^{2+} threshold for Ca^{2+} uptake by MCU [5,7]. Kamer and Mootha now extend the gating of MCU activity to MICU2. Using MICU1 and MICU2 knockout cells, they show that the functions of MICU1 and MICU2 are not redundant, with MICU2 requiring the presence of MICU1 to gate MCU. In a parallel study, Mootha and colleagues showed that EMRE mediates the interaction of MICU1 with MCU [4]. The MCU complex is illustrated in Fig 1. Neither the Ca^{2+} affinity of each MICU1 and MICU2 EF hand nor the reason for MCU gating by two sensor proteins are known at present. However, depending on the Ca^{2+} affinity of the EF hands, two Ca^{2+} sensors functioning in series will sharpen the threshold for MCU $[\text{Ca}^{2+}]_c$ sensing and Ca^{2+} uptake to better discriminate between $[\text{Ca}^{2+}]_c$ spikes of different amplitudes (similar to allostery in enzyme action).

While all studies agree on the gate-keeping function of the MICUs at subthreshold $[\text{Ca}^{2+}]_c$, there is less agreement about the localization of MICU1 and the function of the MICUs EF hands at high $[\text{Ca}^{2+}]_c$ [4–7]. One study concluded that MICU1 resides in the mitochondrial matrix and senses matrix $[\text{Ca}^{2+}]$ [7]. Mutation of the MICU1 EF hands (MICU1^{EFmut}) relieved the inhibition of MCU at subthreshold $[\text{Ca}^{2+}]_c$, as was found by deletion of MICU1, suggesting that Ca^{2+}

binding to the EF hands may induce a MICU1 conformation that is required for the gating function of MICU1 at subthreshold $[\text{Ca}^{2+}]_c$. Measurement of mitochondria Ca^{2+} uptake at $> 10 \mu\text{M}$ $[\text{Ca}^{2+}]_c$ led to the conclusion that the EF hands have no role in the cooperative dependence of mitochondrial Ca^{2+} influx on $[\text{Ca}^{2+}]_c$ [6,7]. The same group recently reported disinhibition of MCU current measured at 5 mM $[\text{Ca}^{2+}]_c$ by expression of MICU1^{EFmut} in mitochondria [6]. Different findings were reported by another study, which concluded that MICU1 resides at the outer surface of the inner mitochondrial membrane (IMM) [5]. Moreover, MICU1^{EFmut} inhibited MCU at subthreshold $[\text{Ca}^{2+}]_c$ similarly to wild-type MICU1, and Ca^{2+} binding to the EF hands had a prominent role in the cooperative dependence of mitochondrial Ca^{2+} influx on $[\text{Ca}^{2+}]_c$ [5].

Kamer and Mootha re-examined this issue in MICU1 and MICU2 knockout, rather than knockdown, cells. At low $[\text{Ca}^{2+}]_c$ load, knockout of either MICU1 or MICU2 increased MCU activity, which was inhibited by expression of the wild-type or the EF hand mutants of the respective MICUs. Most strikingly, in MICU1 knockout cells, the expression of MICU1^{EFmut}, but not of MICU2^{EFmut}, abolished mitochondrial Ca^{2+} uptake at low and high $[\text{Ca}^{2+}]_c$ loads. In addition, in MICU2 knockout cells, the expression of MICU2^{EFmut} or MICU1^{EFmut} abolished mitochondrial Ca^{2+} uptake at low and high $[\text{Ca}^{2+}]_c$ loads. The EF hands of the MICUs are not equivalent, as mutation of a single EF hand only partially inhibits Ca^{2+} uptake, with mutation of EF hand 2 in MICU1 and EF hand 1 in MICU2 had more prominent effect.

Figure 1. The uniporter complex is composed of the pore forming mitochondrial Ca^{2+} uniporter (MCU), MICU1 and MICU2, which are connected by essential MCU regulator (EMRE).

In the resting state, EMRE recruits MICU1 and MICU2 to MCU to keep MCU in the closed state. Modest increase in $[\text{Ca}^{2+}]_c$ above about 2–3 μM results in binding of Ca^{2+} to EF hand 2 of MICU1 and EF hand 1 of MICU2 to increase MCU open probability without disrupting the complex. Higher, pathological increase in $[\text{Ca}^{2+}]_c$ results in Ca^{2+} binding to all EF hands locking MCU in the open state resulting in large mitochondrial Ca^{2+} accumulation. Binding of Ca^{2+} to all EF hands may partially dissociate the MCU complex, releasing MICU1 and MICU2.

These findings suggest that both MICU1 and MICU2 function as Ca^{2+} sensors of MCU. Moreover, Ca^{2+} binding to MICU1 and MICU2 EF hands regulates MCU activity and mitochondrial Ca^{2+} uptake at both low and high $[\text{Ca}^{2+}]_c$ loads. These finding should be contrasted with the recent report that expression of the MICU1^{EFmut} did not inhibit MCU current measured in mitoplasts obtained from transfected HeLa cells [6]. Several experimental differences can account for the difference between the two studies. Expression of the MICU1^{EFmut} increases expression of wild-type MICU1, resulting in markedly abrogated inhibition by the EF hand mutant [8]. The MCU current in mitoplasts was measured by exposing the cytoplasmic face of the IMM to 5 mM Ca^{2+} [6]. It is possible that such high Ca^{2+} concentration overcomes the inhibition of MCU by MICU1^{EFmut}. It is also possible that the high Ca^{2+} used to measure the current might have prevented binding of MICU1^{EFmut} to EMRE and MCU. Finally, knockout of MICU1 resulted in a smaller MCU complex [4], raising the possibility that MICU1 knockout disrupted the intact MCU complex, preventing mitochondrial Ca^{2+} influx. Obviously, further studies are required to reconcile several of these different observations.

It is clear from the discussion above that the field is still in flux. However, all studies agree that MICU1 and MICU2 function as the MCU gatekeepers and that Ca^{2+} binding to their EF hands regulates their gate-keeping function. Ca^{2+} binding to the EF hands likely plays a role at both low and high $[\text{Ca}^{2+}]_c$ loads. Figure 1 attempts to model the role of MICU1 and MICU2 in regulating the function of MCU. MICU2 interacts with MICU1, and both are recruited to MCU by EMRE. At basal $[\text{Ca}^{2+}]_c$, Ca^{2+} -free MICU1 and MICU2 inhibit MCU function. When $[\text{Ca}^{2+}]_c$ increases just above the MCU threshold, the second MICU1 EF hand and first MICU2 EF hand bind Ca^{2+} , resulting in MICUs conformation that exert physiological activation of MCU and mitochondrial Ca^{2+} influx. At very high and sustained increases in $[\text{Ca}^{2+}]_c$, all EF hands bind Ca^{2+} to fully activate MCU possibly by dissociation of the MICUs from the MCU complex, since knockout of the MICUs does not prevent mitochondrial Ca^{2+} influx.

Molecular and genetic analysis of the components of MCU complex in cell physiology is now underway. The recent analysis of MCU knockout mice did surprisingly not reveal major effects on basal metabolism of the animals [9]. However, the MCU knockout mice are smaller and exhibit

marked impairment in performing strenuous work. On the other hand, altered function of MICU1 is associated with disease states. Endothelial cells obtained from patients with cardiovascular disease have normal level of MCU but reduced level of MICU1 and increased MCU current [6]. Loss-of-function mutations in MICU1 are associated with brain and muscle disorders in humans [10]. In both cases, the mitochondria maintain high basal $[\text{Ca}^{2+}]_m$ and Ca^{2+} uptake [6,10]. These findings point to alteration in MICU1 gating of MCU activity and thus uncontrolled mitochondrial Ca^{2+} load as a primary mechanism by which cell stressors cause cell death.

The findings of Kamer and Mootha raise several critical questions. For example, the residence site of the MICUs in the mitochondria is not fully settled. Similarly, the role of the EF hands in the highly cooperative activation of mitochondrial Ca^{2+} influx by $[\text{Ca}^{2+}]_c$ and in Ca^{2+} influx at high $[\text{Ca}^{2+}]_c$ load need further clarification. Future studies are needed to clarify the specific functions of MICU1 and MICU2, the functions of each EF hand, the way in which MICU1 and MICU2 communicate, and to determine the Ca^{2+} binding affinity of each EF hand, just to name a few.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Drago I, Pizzo P, Pozzan T (2011) *EMBO J* 30: 4119–4125
2. Raffaello A, De Stefani D, Sabbadin D *et al* (2013) *EMBO J* 32: 2362–2376
3. Plovanich M, Bogorad RL, Sancak Y *et al* (2013) *PLoS ONE* 8: e55785
4. Sancak Y, Markhard AL, Kitami T *et al* (2013) *Science* 342: 1379–1382
5. Csordas G, Golenar T, Seifert EL *et al* (2013) *Cell Metab* 17: 976–987
6. Hoffman NE, Chandramoorthy HC, Shamu-gapriya S *et al* (2013) *Cell Rep* 5: 1576–1588
7. Mallilankaraman K, Doonan P, Cardenas C *et al* (2012) *Cell* 151: 630–644
8. Kamer K, Mootha V (2014) *EMBO Rep* 15: 299–307
9. Pan X, Liu J, Nguyen T, Liu C *et al* (2013) *Nat Cell Biol* 15: 1464–1472
10. Logan CV, Szabadkai G, Sharpe JA *et al* (2014) *Nat Genet* 46: 188–193