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Reliance of ER-mitochondrial calcium signaling on mitochondrial EF-hand Ca^{2+} binding proteins. Miros, MICUs, LETM1 and solute carriers

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Abstract

Endoplasmic reticulum (ER) and mitochondria are functionally distinct with regard to membrane protein biogenesis and oxidative energy production, respectively, but cooperate in several essential cell functions, including lipid biosynthesis, cell signaling and organelle dynamics. The interorganellar cooperation requires local communication that can occur at the strategically positioned and dynamic associations between ER and mitochondria. Calcium is locally transferred from ER to mitochondria at the associations and exerts regulatory effects on numerous proteins. A common Ca^{2+} sensing mechanism is the EF-hand Ca^{2+} binding domain, many of which can be found in proteins of the mitochondria, including Miro1&2, MICU1,2&3, LETM1 and mitochondrial solute carriers. Recently, these proteins have triggered much interest and were described in reports with diverging conclusions. The present essay focuses on their shared features and established specific functions.

Keywords

endoplasmic reticulum; IP_3 receptor; calcium; mitochondria; EF-hand

Introduction

Mitochondria are strategically distributed in cells, which is attained by transport along microtubules and anchorage to specific sites in mammalian paradigms. A large fraction of mitochondria seems to be positioned close to the ER in live cells [1,2]. The close association of ER and mitochondria is secured by interorganellar tethers, which are formed by a variety of proteins including mitofusin 2, IP_3 receptors-Grp78-VDAC and the ERMES complex in

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yeast [1,2]. Furthermore, the ER membrane and outer mitochondrial membrane (OMM) in the areas of associations have distinctive lipid and protein compositions [3,4]. Diversity in the interface morphology and composition suggests functional specialization. Indeed, recent evidence indicates that the ER-mitochondrial interaction sites are important for various aspects of metabolism, signaling and membrane dynamics. One of the established functions of the ER-mitochondrial interface is to locally transport Ca^{2+} between ER and mitochondria (Figure 1).

Ca^{2+} is a universal signaling entity that exerts regulatory effects via binding to specific proteins. Effectors of Ca^{2+} in the cytoplasmic compartment, including the ER and OMM surface, often involve a ubiquitous Ca^{2+} binding protein, calmodulin (CaM). CaM either directly associates with effectors like the IP3Rs and RyRs or activates enzymes, including kinases and phosphatases (CaM kinase and calcineurin) to mediate Ca^{2+} -induced functional responses. Other effectors are modified by enzymes which directly bind Ca^{2+} , like the Ca^{2+} -activated protease, calpain. The third group of effectors bind Ca^{2+} themselves. Inside the mitochondria, this mechanism of Ca^{2+} regulation dominates, though Ca^{2+} -activated enzymes including PDH phosphatase, calpain and perhaps CaM kinase, may also mediate Ca^{2+} -regulation. Since CaM, CaM kinase/calpain and cytoplasmic enzymes cannot shuttle across the OMM and inner mitochondrial membrane (IMM), they may only confer Ca^{2+} sensitivity to residents of the IMM and matrix when effectively targeted to the mitochondrial interior.

Many of the above described Ca^{2+} sensing mechanisms rely on a highly conserved Ca^{2+} binding motif, the EF-hand. EF-hands are also employed in Ca^{2+} buffer proteins that provide the bulk of the Ca^{2+} buffering capacity in the cytoplasm and ER lumen but are not relevant in the mitochondrial matrix, where Ca^{2+} is primarily bound to cardiolipin or phosphate. The EF-hand motif is defined by its helix-loop-helix secondary structure and by the amino acid residues presented by the loop to bind Ca^{2+} [5]. The amino acids are semi-conserved in the canonical EF-hands but several non-canonical EF-hands exist, which bind Ca^{2+} by different coordination mechanisms. When the helix-loop-helix formation is inserted into a protein it is specifically suited to mediate vast Ca^{2+} -induced conformational changes (prominently exemplified in CaM [6]). Although EF-hand Ca^{2+} binding proteins have been extensively characterized in the past decades, their presence and functional significance in the mitochondria have been seldom considered. However, several recently identified proteins central to ER-mitochondrial communication and mitochondrial function have EF-hands, and many of them have non-canonical structure and are exposed to $[\text{Ca}^{2+}]$ nanodomains (see Table 1). Thus, it is of great significance, and is the primary goal of this review, to create an inventory of these proteins and summarize their functions in ER-mitochondrial calcium signaling.

ER-mitochondrial calcium signaling

Various signals of the cell surface relay their effects to cell function by mobilizing Ca^{2+} stored in the intracellular Ca^{2+} store presented by the endoplasmic or sarcoplasmic reticulum (ER/SR). These organelles maintain a 1000-fold higher $[\text{Ca}^{2+}]$ than the cytoplasm and have Ca^{2+} release channels, the IP₃ receptors (IP3Rs), and the ryanodine receptors (RyRs). Thus,

upon activation of the IP3R or RyR, the cytoplasmic $[Ca^{2+}]$ displays rapid increases in the 100nM to 1 μ M range, which are often manifested as frequency-modulated $[Ca^{2+}]$ oscillations. The bulk cytoplasmic $[Ca^{2+}]$ oscillations represent critical control for a variety of processes. Importantly, in the close vicinity of IP3Rs and RyRs short-lasting >10 μ M $[Ca^{2+}]$ microdomains occur during channel opening. Thus, due to the distribution of mitochondria close to the ER/SR, Ca^{2+} released from the ER/SR exposes the adjacent mitochondrial surface to a $[Ca^{2+}]$ rise in the 10 μ M range [7,8] that is sufficient to rapidly activate the low affinity IMM Ca^{2+} uptake mechanism, the uniporter [9] (Figure 1). The ensuing $[Ca^{2+}]$ increase in the mitochondrial matrix is relevant for activation of the Ca^{2+} -sensitive matrix dehydrogenases to stimulate ATP production and also for opening of the permeability transition pore to commit the cells to death under stress conditions. However, along the pathway of Ca^{2+} transfer from ER to the mitochondria, Ca^{2+} has many other targets, many of which carry EF hands or bind CaM, in the ER membrane, cytoplasm, OMM, IMS, IMM as well as in the mitochondrial matrix (Figure 1). These targets include essentially every Ca^{2+} channel and pump (IP3R and RyR, VDAC, MICU1, SERCA), providing the basis for homeostatic regulation of $[Ca^{2+}]$, but also include a host of Ca^{2+} -regulated mitochondrial solute carriers and enzymes involved in overall ion balancing and in metabolism.

Mitochondrial EF-hand Ca^{2+} binding proteins

A recently established inventory of mitochondrial proteins, the MitoCarta [10] has provided a comprehensive list of the mitochondrial EF-hand Ca^{2+} binding proteins: Rhot 1 (MIRO1), Rhot 2 (MIRO2), Cbara1 (MICU1), efha1 (MICU2), LETM1, mtGPD2 (mtGPDH), AGC1, AGC2, SCAmC1, SCAmC2, SCAmC3, SCAmC-L1, Mitocalcyn, Myosin Regulatory Light Chain 10 (Table 1). In addition, subfractions of some other EF-hand proteins, like S100A1 and calpain were reported in the mitochondria. Strikingly, several proteins listed above have been described as central to ER-mitochondrial calcium signaling and dynamics. Therefore, it is important to summarize their Ca^{2+} regulation and function in a systematic manner. Previous reviews have emphasized the Ca^{2+} regulation of several mitochondrial dehydrogenases, by mechanisms that appear to not involve EF-hands, and its impact on mitochondrial metabolism [11]. Here we focus on the EF-hand containing mitochondrial proteins. The EF-hand Ca^{2+} binding domain sequence and mitochondrial localization of each protein are listed in Table 1, the tissue specific protein abundance for each is shown in Figure 2, whereas their function, knockout/knockdown (KO/KD) phenotypes and human disease relevance are listed in Table 2.

MIRO1 and 2. Ca^{2+} sensitive anchors of mitochondria to the motor complexes

MIRO1/2 and their orthologs are anchored by a C-terminal transmembrane domain to the OMM and contain two GTPase domains flanking a pair of EF-hand Ca^{2+} binding motifs exposed to the cytoplasm [12–14]. Crystal structure studies reported two additional ‘hidden’ EF hands, each paired with a canonical EF hand [15]. MIRO1/2 and their orthologs form complexes with several proteins, including the (1) adaptor proteins TRAK1/2 and their orthologs to anchor mitochondria to microtubular motor proteins, kinesin and dynein

[13,16,17], (2) mitofusins 1/2, which are critical for OMM fusion [18], (3) the PINK1 kinase [19], (4) ARM CX/ARMC10, mitochondria-bound regulators of mitochondrial dynamics [20], and (5) ERMES, the yeast ER-mitochondrial tethering complex [21–23]. These interactions are central to mitochondrial motility and fusion fission dynamics but some additional functions of MIRO1/2, including a role in ER-mitochondrial interactions have also been proposed.

At low $[Ca^{2+}]$, MIRO1/2 facilitate mitochondrial movements and mitochondrial elongation even if their EF-hands are disabled [24]. These functions are dependent on functional GTPase domains [24]. However, MIRO1/2's EF-hands are required for the Ca^{2+} -induced inhibition of mitochondrial movement along the microtubules [24–26]. The contribution of the EF-hands likely supports retention of mitochondria close to ER Ca^{2+} release and allows homeostatic redistribution of the mitochondrial ATP source and Ca^{2+} buffering dependent on the spatial pattern of cellular activities [27]. Ca^{2+} controls the assembly of the MIRO/TRAK/kinesin complex [25,26,28] and its association with ARM CX/ARMC10 [20]. MIRO is also subject of phosphorylation by PINK that activates proteasomal degradation of MIRO in a Parkin-dependent manner [19].

The yeast MIRO ortholog, Gem1p does not seem to confer Ca^{2+} sensitivity to mitochondrial motility. However, loss of Gem1p causes mitochondrial morphology defects [14]. Furthermore Gem1p is associated with the ERMES complex [23,29], though ERMES is not dependent on the presence of Gem1p [30,31]. Gem1p has been spatially and functionally linked to ER-associated mitochondrial division [21] and to phosphatidylserine transport from ER to mitochondria [22] (but different results in [30]). Although mitochondrial inheritance was not dependent upon Ca^{2+} binding by the two EF-hands of Gem1p, a functional N-terminal EF-hand motif was critical for stable expression of Gem1p and for the association of Gem1p with the ERMES complex in vivo [22,32]. The mammalian counterparts of the ERMES complex remain elusive as well as the relevance of MIRO1/2 for the ER-mitochondrial tethers.

MICU1, 2 (3). Ca^{2+} sensitive regulators of the calcium uniporter, MCU

Multiple lines of evidence supports that MICU1 and MICU2 are localized to the IMS and are associated with the IMM [33,34]. The IMM association seems to include (1) hydrophobic stretches at the N terminus that can interact with membrane lipids [33,35] and (2) protein interactions with transmembrane proteins of the IMM, including a domain of MICU1 that associates with EMRE [36], and a domain binding directly to MCU [28,37–39]. MICU1/2 together with MCU, EMRE and several other proteins form the mitochondrial Ca^{2+} uniporter complex [36]. Distribution of the uniporter at the ultrastructural level has not been documented but its localization to the areas of the IMM close to the ER-mitochondrial associations is supported by maximal activation of the uniporter during ER Ca^{2+} release [40] at least in some cell types.

The OMM shows higher Ca^{2+} permeability than the IMM, allowing MICU1/2 to sense and respond to fluctuations of the cytoplasmic $[Ca^{2+}]$. At submicromolar $[Ca^{2+}]_c$ levels, MICU1/2 helps to keep the uniporter's pore, formed by the MCU, closed [33,41,42].

Mutations that cripple Ca^{2+} binding of the EF-hands do not interfere with MICU's "gatekeeping" function [33,34]. Based on bilayer electrophysiology studies carried out in the absence of Ca^{2+} and Mg^{2+} with recombinant proteins, Patron et al. reported that MICU2 can suppress the MCU monovalent conductance, whereas MICU1 can't. The results were interpreted that MICU1 and MICU2 had opposite effects on MCU [37]. However, previous studies have shown that mitochondria that have normal gatekeeping function become permeable for monovalent cations after removal of Mg^{2+} [43,44]. Furthermore, several tissues seem to express little MICU2 (Figure 2) and still have the mitochondrial uniporter closed at low $[\text{Ca}^{2+}]_c$ based on studies of isolated mitochondria. Thus, in vivo, MICU1 might be competent to provide "gatekeeping" function.

At high $[\text{Ca}^{2+}]_c$, MICU1 supports cooperative activation of the uniporter [33,34,37]. This effect is eliminated by mutation of MICU1's EF-hands and is likely mediated by Ca^{2+} binding to the EF-hand(s) [28,33]. MICU2 seems to be less effective in supporting the activation of the uniporter than MICU1 [37], which might be due to its distinct EF-hand Ca^{2+} binding domain amino acid sequences (EF1, Table 1). The EF-hand mediated cooperative activation of the uniporter seems to be particularly important for local Ca^{2+} delivery between ER and mitochondria, since the IP3R or RyR-mediated Ca^{2+} release produces very transient high $[\text{Ca}^{2+}]_c$ exposure of the mitochondria.

It has to be mentioned that Foskett, Madesh et al proposed that MICU1 is located in the mitochondrial matrix, binds Ca^{2+} in unstimulated condition and mediates only a "gatekeeping" function [42,45]. This model does not seem to be consistent with the results of several groups [28,33,34,37] and the present brief review format precludes detailed discussion regarding potential sources of their different views. MICU3 is primarily expressed in the nervous system and was excluded from this discussion because its function has not been evaluated, yet.

LETM1. Ca^{2+} -sensitive K^+ (Ca^{2+} ?) carrier

LETM1 is a transmembrane protein of the IMM, which exposes its EF-hand in the matrix [46]. LETM1 is a carrier protein that has been described as a K^+/H^+ exchanger [47,48]. Recent results from the Clapham lab indicated that LETM1 can also mediate $\text{Ca}^{2+}/\text{H}^+$ exchange [49,50]. These divergent results have been recently reviewed [51]. However, the significance of the EF-hand Ca^{2+} binding domains is unclear.

Ca^{2+} sensitive solute carriers. AGC1, and 2, SCaMC1, 2 and 3

Both families of the solute carriers display the IMM spanning transmembrane domains at the C terminus and EF-hands at the N terminus. Furthermore, all these proteins support mitochondrial metabolism by mediating the exchange of various intermediates and ATP [52] (Table 2). They are inactive unless are stimulated by a $[\text{Ca}^{2+}]_c$ rise.

The number and arrangements of the EF-hands are different for AGC1/2 and SCaMCs. A recent structural study has demonstrated that SCaMCs, like CaM, have 2 pairs of EF-hands, which upon binding of Ca^{2+} undergo a rearrangement that weakens the interaction between the N terminus and the C terminal transport domain [53].

During propagations of $[Ca^{2+}]_c$ signals to the mitochondria, activation of the solute carriers can effectively complement the Ca^{2+} -induced activation of the Ca^{2+} sensitive matrix dehydrogenases, including the pyruvate dehydrogenase, the isocitrate dehydrogenase and oxoglutarate dehydrogenase to stimulate oxidative metabolism. In addition, due to the localization of their EF-hands to the IMS and to their sensitivity to submicromolar $[Ca^{2+}]_c$ increases [54], the solute carriers can also respond to physiological global $[Ca^{2+}]_c$ elevations which fail to activate the uniporter.

Similar to the AGCs and SCaMCs, the mtGPDH also localizes to the IMM and mediates the transport of metabolic intermediates (Table 2). Furthermore, the EF-hands of mtGPDH are localized to the IMS and are sensitive to submicromolar increases of $[Ca^{2+}]_c$. Ca^{2+} lowers the K_m for glycerol-3-phosphate by 2–3 fold [55]. Thus, mitochondrial metabolism can be stimulated through mtGPDH activation during both local high $[Ca^{2+}]_c$ exposure and global $[Ca^{2+}]_c$ elevations.

Outlook

Mitochondrial EF-hand proteins are central to the proper decoding of the $[Ca^{2+}]$ fluctuations to control both metabolism and stress responses. Therefore, impaired Ca^{2+} sensing by mitochondrial EF-hand proteins is expected to present a considerable risk for any organism. Indeed, gene ablation studies in animals and gene polymorphisms in humans have provided evidence that these proteins are vital for health (Table 2). Thus one of the major challenges is to explore the possible link between mutations of the various mitochondrial EF-hand Ca^{2+} binding proteins and human disease, and to determine how mutations selectively affecting the EF hands of these proteins alter cell function. The rapidly expanding clinical genetics is expected to be helpful to assign further patient populations to mitochondrial EF-hand proteins in the future. Mitochondrial EF-hand proteins show considerable sequence diversity in the Ca^{2+} binding domains (Table 1), the relevance of which in terms of function remains to be determined. Analysis of the Ca^{2+} binding kinetics, affinity and specificity of the various mitochondrial EF-hands will also help to identify specific drug targets that might become useful for the therapy of diseases that so far lack specific and effective treatment. An additional level of complexity of the mitochondrial EF-hands is due to their exposure to the different and insufficiently characterized Ca^{2+} environments of the mitochondrial surface, the IMS and the matrix. Furthermore, even in the case of a single compartment, the Ca^{2+} exposure might be dependent on the distribution of EF-hands relative to potential local Ca^{2+} sources. For example, EF-hands close to the region of ER-mitochondrial associations in each compartment can be exposed to >10-fold larger local $[Ca^{2+}]$ than the global concentration. Therefore understanding of the operation of the different mitochondrial EF-hands will require mapping of their intramitochondrial distribution and direct measurement of the local $[Ca^{2+}]$ that they see.

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Figure 1. Localization and function of the mitochondrial EF-hand Ca^{2+} binding proteins
EF-hand proteins are marked by red symbols and the location of their EF-hands is indicated in blue. The scheme also depicts the molecules mediating ER and mitochondrial Ca^{2+} fluxes at an area of ER-mitochondrial association. The shades of gray represent the $[\text{Ca}^{2+}]$ during IP3R-mediated Ca^{2+} release (dark gray, 100–500 μM , white: 100nM).

	cerebrum	cerebellum	brainstem	spinal cord	kidney	liver	heart	skeletal muscle	adipose	stomach	small intestine	large intestine	testis	placenta	pooled
Miro1	10	9	10	9	9	9	0	9	9	9	9	9	10	9	10
Miro2	10	9	9	9	9	8	7	8	9	9	9	9	9	9	10
Micu1	9	9	9	8	0	9	0	9	8	8	9	9	9	9	10
Micu2	0	8	0	0	0	0	0	0	8	8	9	9	9	8	10
Micu3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Letm1	10	10	11	10	11	10	10	10	10	10	11	11	10	10	12
mtGPDH	11	10	11	10	11	10	0	11	11	10	11	10	11	10	12
AGC1	11	11	11	11	11	10	11	12	10	10	10	10	10	11	12
AGC2	10	10	11	10	11	11	11	12	9	10	10	10	10	10	12
SCaMC1	8	9	0	0	0	0	0	0	9	9	11	10	8	10	11
SCaMC2	9	8	9	10	0	8	0	0	8	0	7	0	0	8	10
SCaMC3	9	0	0	8	8	0	0	0	8	7	0	0	0	0	9

Figure 2. Relative abundance of the EF-hand Ca^{2+} binding proteins listed in the MitoCarta in different tissues

Numbers were taken directly from MitoCarta (Mouse) and refer to relative protein abundance. Protein abundance was determined by MS total peak intensity, which can only be used to compare between tissues for a given protein [10]. It should be noted that (1) each tissue reflects only a single replicate; (2) it was estimated that detection is possible for only ~90% of the proteins per tissue because the methods lead to a bias against detection of low expression levels [10], '0' should not be strictly taken to indicate no expression.

Table 1

EF motif sequence and localization of the mitochondrial EF-hand Ca²⁺-binding proteins

Protein Name <i>Gene Name</i>	EF motifs 123456789012	Pattern Match *	NCBI conserved domains	Subcellular Localization
MIRO1 <i>Rhot1</i>	EF1> DQDNDGTLNDAE	Y	N/A	Protein: OMM transmembrane EF-hand: cytoplasm
	EF2> DLDRDCALSPDE	N		
MIRO2 <i>Rhot2</i>	EF1> DQDLQALSDEE	N	N/A	
	EF2> DQDRDGALSPVE	Y		
MICU1 <i>Micu1</i>	EF1> DLNGDGEVDMEE	Y	EF-hand_8 N/A	Protein: IMM associated EF-hand: IMS
	EF2> DCDGNGELSNKE	Y		
MICU2 <i>Micu2</i>	EF1> DTDGNEMIEKRE	N	N/A EF-hand_8	Protein: IMS? EF-hand: IMS?
	EF2> DLDGDECLSHEE	N		
MICU3 <i>Micu3</i>	EF1> DTDGNEMVDKKE	N	EF-hand_8 EF-hand 8	Protein: ? EF-hand: ?
	EF2> DVDKDDQLSYKE	Y		
LETM1 <i>Letm1</i>	EF1> DYSEDLQEIKKE	N	N/A EF-hand 8	Protein:IMM transmembrane EF-hand: matrix
	EF2> DENKDGKVNIDD	Y		
mtGPDH <i>Gpd2</i>	EF1> DADQKGFTIVD	N	EF-hand_7 pair	Protein: IMM associated EF-hand: IMS
	EF2> DLNKNQGVELNE	Y		
AGC1 <i>Slc25a12</i>	EF1> EVDGERYMTPED	N	EF1&2: EF-hand_7 EF2&3: EF-hand_7 EF4: N/A	Protein:IMM transmembrane EF-hand: IMS
	EF2> DQTKDGLISYQE	N		
	EF3> DKSGNGEVTFEN	N		
	EF4> DKSKSGMISGLD	Y		
AGC2 <i>Slc25a13</i>	EF1> EKNGEFFMSPND	N	EF-hand_7 pair [†]	Protein:IMM transmembrane EF-hand: IMS
	EF2> DQTKDGLISFQE	N		
SCaMC1 <i>Slc25a24</i>	EF1> DRNGDGVVDIGE	Y	EF1&2: EF-hand_7 EF3&4: EF-hand_7	Protein:IMM transmembrane EF-hand: IMS
	EF2> DVNKDGKLDLEE	Y		
	EF3> DKNNDGKIEASE	Y		
	EF4> DVDGTMTVDWNE	N		
SCaMC2 <i>Slc25a25</i>	EF1>	N	EF1&2: EFh EF2&3: EF-hand_7 EF3&4: EF-hand_7	Protein:IMM transmembrane EF-hand: IMS
	SVFIPSQE [‡]	N		
	EF2> DKDLGQLDFEE	N		
	EF3> DKKNDGRIDAQE	N		
	EF4> DKNGTMTIDWNE			
SCaMC3 <i>Slc25a23</i>	EF1> DSNKDGRVDVHE	Y	EF1&2: EF-hand_7 EF3&4: EF-hand_7	Protein:IMM transmembrane EF-hand: IMS
	EF2> DADPDGGLDLEE	N		
	EF3> DRNQDGHIDVSE	Y		
	EF4> DRDGTMTIDWQE	N		
SCaMC-L1(mm) [§] <i>4930443G12Rik</i>	EF1> DHNGDGVVDITE	Y	EF1&2: EFh EF3&4: EFh [§]	Protein:IMM transmembrane EF-hand: IMS
	EF2> DSNADSGLDFEE	N		
	EF3> DKNDGVIDASE	Y		

Protein Name <i>Gene Name</i>	EF motifs 123456789012	Pattern Match [*]	NCBI conserved domains	Subcellular Localization
	EF4> DFDGSMTVDWDE	N		
S100A1 <i>S100a1</i>	EF1>	N	EF-hand_7 pair [†]	Protein:? EF-hand:?
	SKEGDKYkLSKKE//	N		
	EF2> DQTKDGLISFQE			
Mitocalcin <i>Efh1</i>	EF1> DAGRDGFIDLME	N	EF-hand_7 pair	Protein:IMM EF-hand:?
	EF2> DEDFDGKLSFRE	N		
Myosin regulatory light chain 10 <i>Myl10</i>	EF1> DQNRDGFIDKED	Y	EFh EFh [¶]	Protein:? EF-hand:?
	EF2> DTEGKGFVKADV	N		

^{*} Based on the Prosite canonical EF-hand motif: D-{W}-[DNS]-{ILVFYW}-[DENSTG]-[DNQGHRK]-{GP}-[LIVMC]-[DENQSTAGC]-x(2)-[DE]

[†] Also S-100 domain superfamily

[‡] Dashes indicate a deletion in the conserved domain alignment.

[§] mm = *Mus musculus*; gene does not exist in humans.

// lower case 'k' represents an inserted lysine, ignored for the pattern match of subsequent amino acids.

[¶] All EF hands also part of FRQ1 'multi-domain'.

Table 2Functional implications of the mitochondrial EF-hand Ca^{2+} binding proteins

Protein	Function	KD/KO phenotype	Human Gene Mutation and Disease
MIRO1, 2	Anchoring motor proteins to mitochondria [13,16,56]	<u>KO yeast (<i>GEM1</i>):</u> <ul style="list-style-type: none"> Collapsed, grape-like mitochondria [14] ER-Mitochondrial associations: fewer, larger area/contact [22] <u>KO fly (<i>dMiro</i>):</u> <ul style="list-style-type: none"> Survival: ↓, Locomotion and neuromuscular junction phenotypes [56] 	
mtGPHD	Converts glycerol-3 phosphate to dihydroxyacetone phosphate coupled to FAD reduction: glycerol-phosphate shuttle (Review:[55])	<u>KO mouse:</u> <ul style="list-style-type: none"> Fertility and postnatal survival: ↓ [55] Metabolic phenotypes: systemic, islets, brown adipose tissue [55] 	<ul style="list-style-type: none"> Intellectual disability [57] (298 bp deletion on chromosome 24 encompassing mtGPHD; gene disruption at 2q24.1 region) Type 2 diabetes exacerbation [58] (c. 2018T>C, c.2069A>C, c. 2071T>C, c.2136C>T)
MICU1, 2	MICU1, 2: Regulate $[\text{Ca}^{2+}]$ threshold and sensitivity for MCU-mediated Ca^{2+} uptake [33,34,37,59]	<u>MICU1 KD human cells:</u> <ul style="list-style-type: none"> Cell death via mitochondrial permeability transition: ↑ [33,59] Oxidative stress: ↑[33,45,59] Endothelial cell migration: ↓[45,59] <u>Micu1 KD mouse liver:</u> <ul style="list-style-type: none"> Mitochondrial Ca^{2+} overload: ↑ [33] <u>Micu1 KD mouse epithelial cells:</u> <ul style="list-style-type: none"> Vascular leakage [45] 	<u>MICU1.</u> <ul style="list-style-type: none"> Skeletal muscle weakness, various neurological symptoms, and peripheral neuropathy [60] (c. 741+1G>A, c.1078-1G>C)
LETM1	Cation exchanger (K^+ , Ca^{2+} in exchange for H^+) (Review: [51])	<u>KO yeast (<i>YOL027</i>):</u> <ul style="list-style-type: none"> Swollen mitochondria [48], mitochondrial matrix $[\text{K}^+]$: ↑[48] <u>KD human cells:</u> <ul style="list-style-type: none"> Fragmentation of mitochondria [61], fewer cristae [62], survival: ↓ [61] <u>KO mouse:</u> <ul style="list-style-type: none"> Early embryonic lethality in homozygous and neuronal metabolic phenotypes in heterozygous mice [61] 	<ul style="list-style-type: none"> Associated with Wolf-Hirschhorn syndrome [46,47] (Hemizygous deletion on chromosome 4 encompassing <i>LETM1</i>)
AGC1, 2	Catalyzes 1:1 exchange of aspartate for glutamate; part of malate/aspartate shuttle (MAS).	<u>Agc1 KO mouse:</u> <ul style="list-style-type: none"> Postnatal neuronal development defects [63] Neurological, neuronal phenotypes [54,64] Heart mitochondria: ↓ Ca^{2+} activation of MAS [65] 	<u>SLC25A12:</u> <ul style="list-style-type: none"> Epilepsy [67] (c. 1058G>A), autism [68] (various SNPs) Global cerebral hypomyelination [69] (c. 1769A>G) <u>SLC25A13:</u>

Protein	Function	KD/KO phenotype	Human Gene Mutation and Disease
		<u>Agc2 KO mouse:</u> <ul style="list-style-type: none"> Liver: metabolic changes [66] 	<ul style="list-style-type: none"> Neonatal Intrahepatic Cholestasis due to Citrin Deficiency (NICCD) [70], Adult-onset type II citrullinemia (CTLN2) [70] (several mutations).
SCaMC-1, 2, 3	Electroneutral exchange of ATP- Mg^{2+} or $HADP^{2-}$ for phosphate (HPO_4^{2-})	<u>SLC25A24 human KD cells:</u> <ul style="list-style-type: none"> Oxidative stress-induced cell death: $\uparrow$ Mitochondrial Ca^{2+} retention: $\downarrow$ [71] <u>Slc25A25 KO mouse:</u> <ul style="list-style-type: none"> Metabolic phenotypes: whole-body level and mouse embryonic fibroblasts [72] <u>Slc25A23 KO mouse:</u> <ul style="list-style-type: none"> Hepatocytes: $\downarrow$ mitochondrial energetic response to glucagon; $\downarrow$ mitochondrial Ca^{2+} retention capacity [73] 	
S100A1	Calcium buffering and sensing (Review: [74])	<u>KO mouse and cardiomyocytes:</u> <ul style="list-style-type: none"> Cardiac response to β-adrenergic stimulation: $\downarrow$ [74], Ca^{2+} homeostasis: disturbed [75] 	