

# Insight of the bioinformation: the discovery of mitochondrial calcium uniporter

生物信息学的启示：线粒体钙单向转运体的发现

Group members: Zhao Qijin, Li Wenwen  
Zhang Rufeng, Wang Lun

2015/01/25



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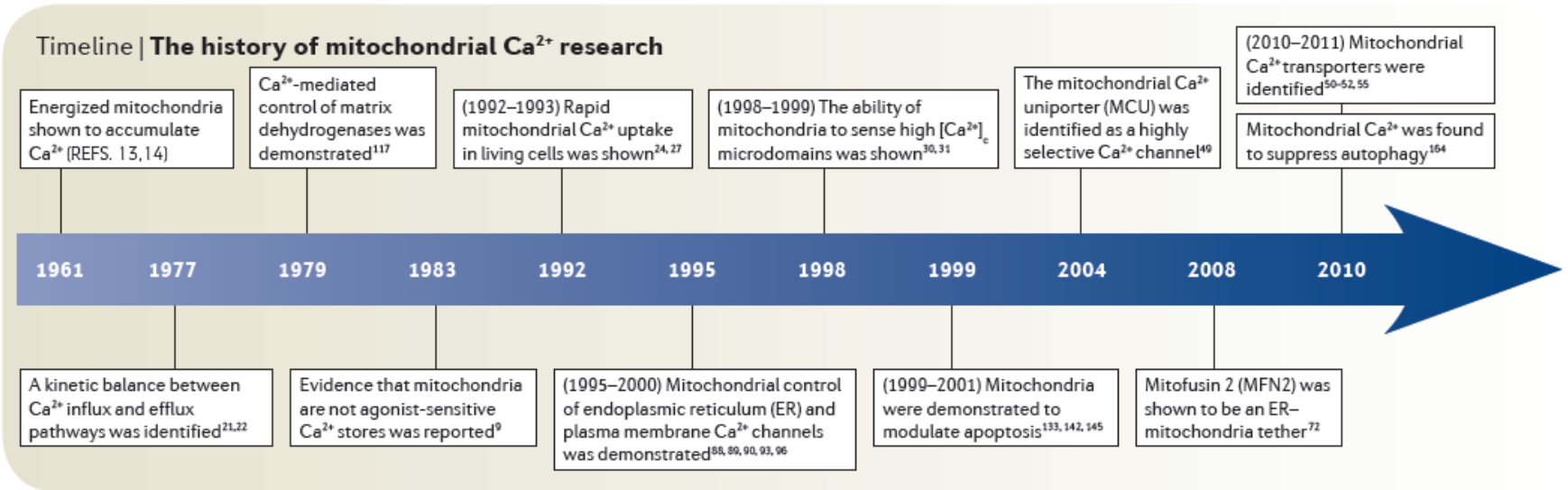
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# The history of mitochondrial calcium research



**Genome-Wide RNAi Screen Identifies Letm1 as a Mitochondrial Ca<sup>2+</sup>/H<sup>+</sup> Antiporter**  
Dawei Jiang *et al.*  
*Science* **326**, 144 (2009);  
DOI: 10.1126/science.1175145

Nat Rev Mol Cell Biol. 2012 Sep;13(9):566-78.

Science. 2009 Oct 2;326(5949):144-7.



# The mysterious molecule buried in the half century

MCU: Mitochondrial calcium uniporter (线粒体钙单向转运体)

## LETTER

doi:10.1038/nature10234

### Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter

Joshua M. Baughman<sup>1,2\*</sup>, Fabiana Perocchi<sup>1,2\*</sup>, Hany S. Girgis<sup>1,2</sup>, Molly Plovanich<sup>1,2</sup>, Casey A. Belcher-Timme<sup>1,2</sup>, Yasemin Sancak<sup>1,2</sup>, X. Robert Bao<sup>1,2</sup>, Laura Strittmatter<sup>1,2</sup>, Olga Goldberger<sup>1,2</sup>, Roman L. Bogorad<sup>3</sup>, Victor Koteliansky<sup>4</sup> & Vamsi K. Mootha<sup>1,2</sup>

## LETTER

doi:10.1038/nature10230

### A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter

Diego De Stefani<sup>1,2\*</sup>, Anna Raffaello<sup>1,2\*</sup>, Enrico Teardo<sup>3</sup>, Ildikò Szabò<sup>3</sup> & Rosario Rizzuto<sup>1,2</sup>

Nature. 2011 Jun 19;476(7360):341-5.

Nature. 2011 Jun 19;476(7360):336-40.



## How to open the scientific secret buried in the half century— Previous research results

### What do we know until we begin the screening of the mitochondrial calcium uptake machine based nearly fifty years?

- 1 ubiquitous expression in mammalian tissues
- 2 predicted transmembrane domains in the primary sequence
- 3 It is a high-capacity, ruthenium-red ( RuR , 钌红 ) -sensitive mitochondrial  $\text{Ca}^{2+}$  uptake machinery
- 4 *Saccharomyces cerevisiae* ( 酿酒酵母 ) lacks a RuR-sensitive mitochondrial  $\text{Ca}^{2+}$  uptake route
- 5 In kinetoplastids ( 动基体类 ) (Trypanosoma ( 锥虫属 ) and Leishmania ( 利什曼原虫 ) ), rapid, uncoupler-sensitive  $\text{Ca}^{2+}$  fluxes to mitochondria were measured.



# How to open the scientific secret buried in the half century— MitoCarta Database

Cell

## A Mitochondrial Protein Compendium Elucidates Complex I Disease Biology

David J. Pagliarini,<sup>1,2,3,9</sup> Sarah E. Calvo,<sup>1,2,3,4,9</sup> Betty Chang,<sup>3</sup> Sunil A. Sheth,<sup>1,2,3,4</sup> Scott B. Vafai,<sup>1,2</sup> Shao-En Ong,<sup>3</sup> Geoffrey A. Walford,<sup>1,2</sup> Canny Sugiana,<sup>5</sup> Avihu Boneh,<sup>5,6</sup> William K. Chen,<sup>1,2</sup> David E. Hill,<sup>7</sup> Marc Vidal,<sup>7</sup> James G. Evans,<sup>9</sup> David R. Thorburn,<sup>5,6</sup> Steven A. Carr,<sup>3,4</sup> and Vamsi K. Mootha<sup>1,2,3,4</sup>\*

112 Cell 134, 112–123, July 11, 2008 ©2008 Elsevier Inc.

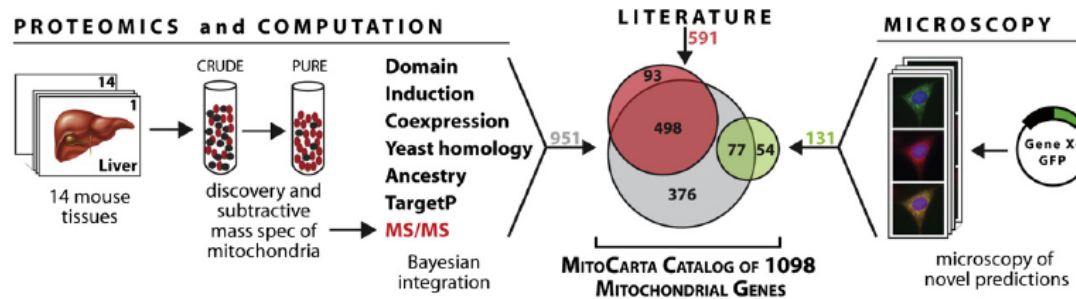


Figure 1. Building a Compendium of Mitochondrial Proteins

The screenshot shows the MitoCarta website homepage. The header includes the Broad Institute logo and navigation links: Partnerships, Contribute, Careers, Contact Us, What is Broad, News and Publications, and For the Scientific Community. The main content area features the title "MitoCarta: An Inventory of Mammalian Mitochondrial Genes" and a brief description: "MitoCarta is an inventory of 1098 mouse genes encoding proteins with strong support of mitochondrial localization. To generate this inventory, we performed mass spectrometry of mitochondria isolated from fourteen tissues, assessed protein localization through large-scale GFP tagging/microscopy, and integrated these results with six other genome-scale datasets of mitochondrial localization, using a Bayesian approach. We also provide the inventory of 1013 human HomoloGene homologs to the mouse MitoCarta collection." A citation is provided at the bottom: "Citation: Pagliarini, D.J., Calvo, S.E., Chang, B., Sheth, S.A., Vafai, S.B., Ong, S.E., Walford, G.A., Sugiana, C., Boneh, A., Chen, W.K., et al. (2008) A mitochondrial protein compendium elucidates complex I disease biology. Cell 134, 112-123. [PubMed: 18614015]"

Cell. 2008 Jul 11;134(1):112-23.



# Mouse MitoCarta and Human MitoCarta

## Mouse MitoCarta: 1098 mitochondrial genes

The MitoCarta mouse inventory is a collection of 1098 nuclear and mtDNA genes encoding proteins with strong support of mitochondrial localization. The table below provides a summary of evidence supporting mitochondrial localization as well as the protein distribution across 14 tissues. The genes are ordered by strength of mitochondrial evidence. More complete information is available for download in file [Mouse.MitoCarta.xls](#). See also [MitoCarta home](#) for a description of the project and [MitoCarta documentation](#) for information on the columns below.

EntrezID	Symbol	Description	Synonyms	Maestro Score	cFDR	Evidence	Tissues
22273	Uqcrc1	ubiquinol-cytochrome c reductase core protein 1	1110032610Rik, MGC97899	42	0%	literature, targetP signal, yeast mito homolog, Rickettsial homolog, mito protein domain, induction+, coexpression+,	all 14

## Human MitoCarta: 1013 mitochondrial genes

The MitoCarta human inventory is a collection of 1013 nuclear and mtDNA genes encoding proteins with strong support of mitochondrial localization based on homology to mouse MitoCarta genes. The table below provides a summary of evidence supporting mitochondrial localization as well as the protein distribution across 14 tissues. The genes are ordered by strength of mitochondrial evidence. More complete information is available for download in file [Human.MitoCarta.xls](#). See also [MitoCarta home](#) for a description of the project and [MitoCarta documentation](#) for information on the columns below.

EntrezID	Symbol	Description	Synonyms	Maestro Score	cFDR	Evidence	Tissues
20916	Sucl2	succinate-Coenzyme A ligase, A					
7384	UQCRC1	ubiquinol-cytochrome c reductase core protein I	D3S3191	42	0%	literature, targetP signal, yeast mito homolog, Rickettsial homolog, mito protein domain, induction+, coexpression+, MS/MS+	all 14
12859	Cox5b	cytochrome c oxidase, subunit b					
3420	IDH3B	isocitrate dehydrogenase 3 (NAD+) beta	FLJ11043, H-IDHB, MGC903	41	0%	literature, targetP signal, yeast mito homolog, Rickettsial homolog, mito protein domain, induction+, coexpression+, MS/MS+	all 14
67680	Sdhb	succinate dehydrogenase complex					
5160	PDHA1	pyruvate dehydrogenase (lipoamide) alpha 1	PDHA, PDHCE1A, PHE1A	41	0%	literature, targetP signal, yeast mito homolog, Rickettsial homolog, mito protein domain, induction, coexpression+, MS/MS+	all 14
66945	Sdha	succinate dehydrogenase complex					
8803	SUCLA2	succinate-CoA ligase, ADP-forming, beta subunit	A-BETA	39	0%	literature, targetP signal, yeast mito homolog, Rickettsial homolog, mito protein domain, induction+, coexpression+, MS/MS+	all 14
1329	COX5B	cytochrome c oxidase subunit Vb	COX5B	39	0%	literature, targetP signal, yeast mito homolog, mito protein domain, induction+, coexpression+, MS/MS+	all 14
17448	Mdh2	malate dehydrogenase 2, NAD (m)					
6390	SDHB	succinate dehydrogenase complex, subunit B, iron sulfur (Ip)	IP, PGL4, SDH, SDHI, SDHIP	38	0%	literature, targetP signal, yeast mito homolog, Rickettsial homolog, mito protein domain, induction+, coexpression+, MS/MS+	all 14
6389	SDHA	succinate dehydrogenase complex, subunit A, flavoprotein (Fp)	FP, SDH2, SDHF	38	0%	literature, targetP signal, yeast mito homolog, Rickettsial homolog, mito protein domain, induction+, coexpression+, MS/MS+	all 14
4191	MDH2	malate dehydrogenase 2, NAD (mitochondrial)	M-MDH, MDH, MGC:3559, MOR1	38	0%	literature, targetP signal, yeast mito homolog, Rickettsial homolog, mito protein domain, induction+, coexpression+, MS/MS+	all 14
1738	DLD	dihydrolipoamide dehydrogenase	DLDH, E3, GC5L, LAD, PHE3	38	0%	literature, targetP signal, yeast mito homolog, Rickettsial homolog, mito protein domain, induction+, coexpression+, MS/MS+	all 14
10229	C007	coenzyme Q7 homolog, ubiquinone (yeast)	CA				

<http://www.broadinstitute.org/pubs/MitoCarta/>



# How to open the secret buried in the half century— **Bioinformatics guided studies**



Vamsi K. Mootha

**Vamsi K. Mootha, MD**

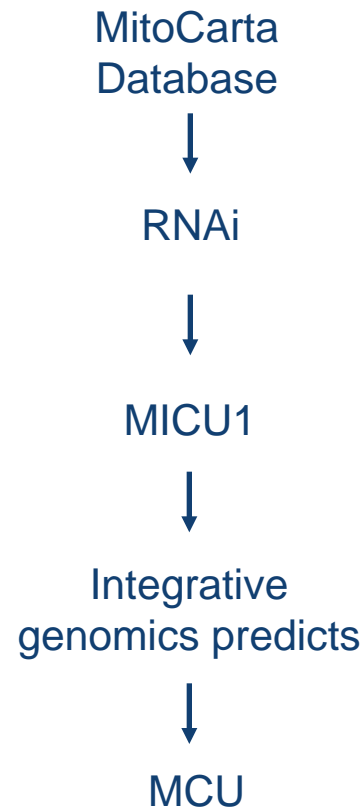
**Investigator**  
Howard Hughes Medical Institute

**Professor**  
Department of Systems Biology,  
Harvard Medical School  
Department of Medicine,  
Massachusetts General Hospital

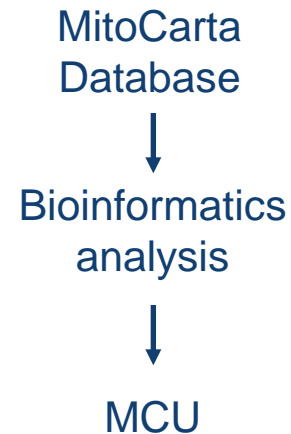
**Institute Member**  
Broad Institute of MIT and Harvard

B.S., Stanford University  
M.D., Harvard Medical School

Joined the Harvard Faculty in 2004



Rosario Rizzuto



University of Padova,  
Padua, Veneto, Italy



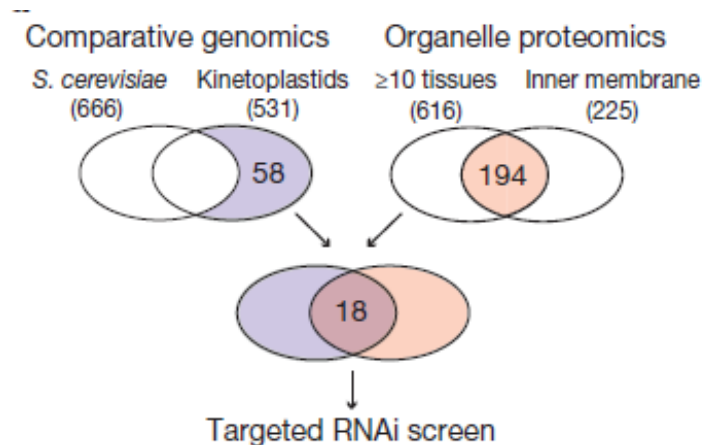


# How to open the scientific secret buried in the half century: The strategy of Mootha VK. Group

On the basis of decades of biochemical characterization, the **Mootha VK.**

**Group** expect to be:

- (1) localized to the mitochondrial inner membrane
- (2) expressed in the majority of mammalian tissues
- (3) to have homologues in vertebrates and kinetoplastids, but not in the yeast *S. cerevisiae*



Nature. 2010 Sep 16;467(7313):291-6.



# The prelude of the main movement: **The discovery of MICU1**

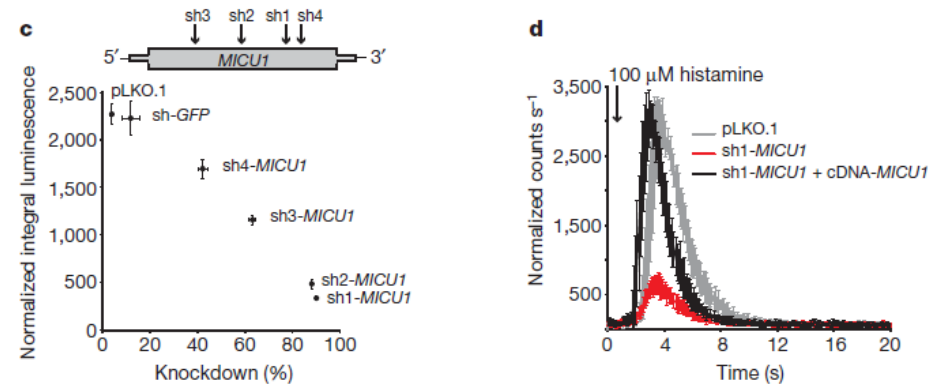
Vol 467 | 16 September 2010 | doi:10.1038/nature09358

nature

## ARTICLES

### **MICU1 encodes a mitochondrial EF hand protein required for $\text{Ca}^{2+}$ uptake**

Fabiana Perocchi<sup>1</sup>, Vishal M. Gohil<sup>1</sup>, Hany S. Girgis<sup>1</sup>, X. Robert Bao<sup>1</sup>, Janet E. McCombs<sup>2</sup>, Amy E. Palmer<sup>2</sup> & Vamsi K. Mootha<sup>1</sup>



### MICU1基因沉默抑制线粒体钙摄取

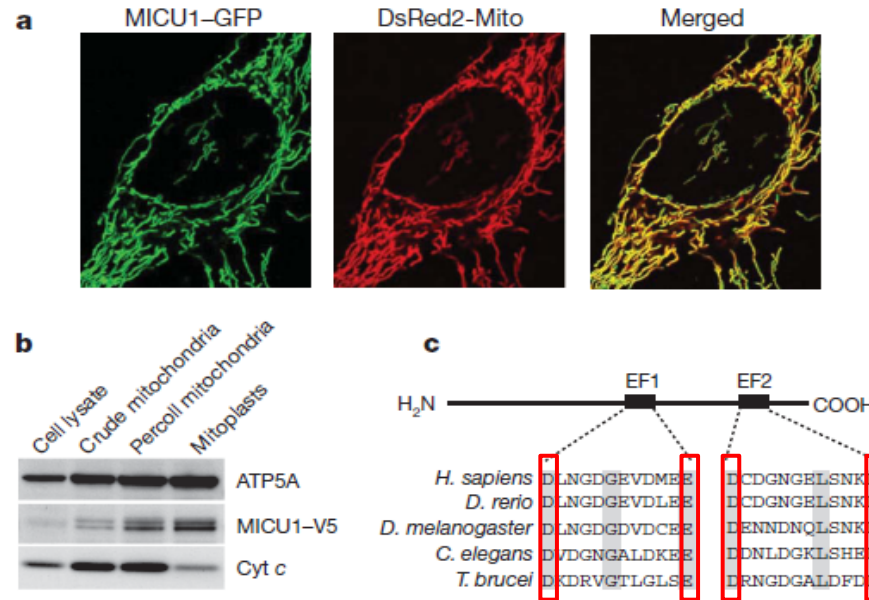
**MICU1: a candidate from the mitochondrial calcium uptake machine by genomics and proteomics**

**MICU1: Mitochondrial calcium uniporter1—It was not the actual mitochondrial  $\text{Ca}^{2+}$  channel**

Nature. 2010 Sep 16;467(7313):291-6.



# MICU1:an EF-hand protein localized to mitochondria



MICU1是一个具有EF-hand结构域的线粒体内膜蛋白

The **EF hand** is a helix-loop-helix structural domain or *motif* found in a large family of calcium-binding proteins.

Acidic residues enriched calcium binding sites : EF-hand

Uniprot

Regions

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Calcium binding <sup>1</sup>	231 - 242	12	1			<a href="#">b Add</a> <a href="#">t BLAST</a>
Calcium binding <sup>1</sup>	421 - 432	12	2			<a href="#">b Add</a> <a href="#">t BLAST</a>

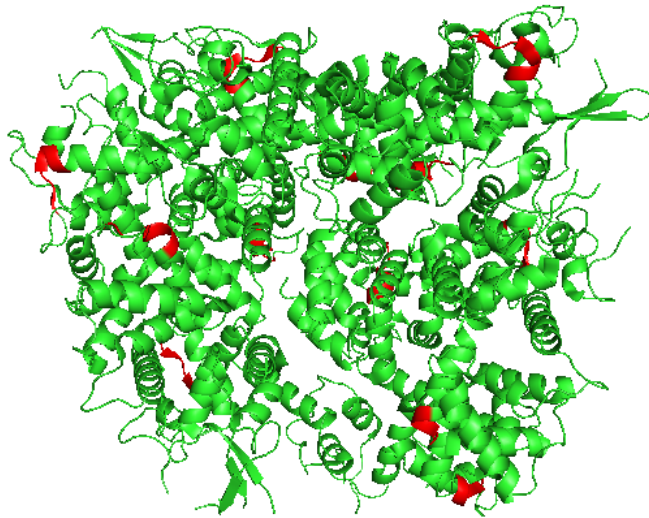
Nature. 2010 Sep 16;467(7313):291-6.



# MICU1:Crystal structures

EF-hand ( $\text{Ca}^{2+}$  free)

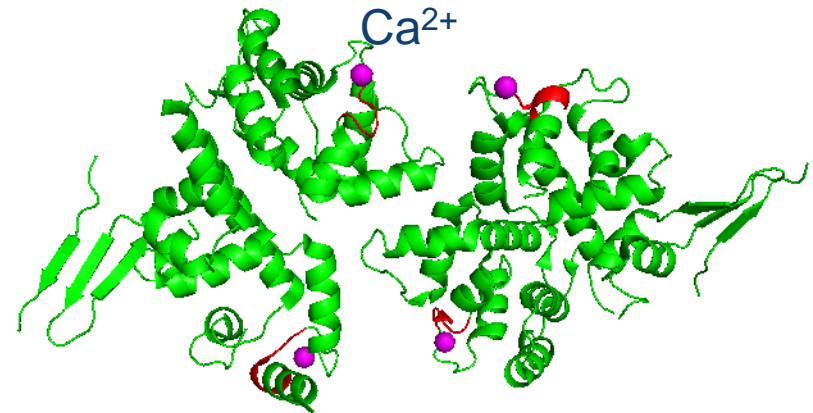
For Educational Use Only



hexamer

EF-hand ( $\text{Ca}^{2+}$  binding)

For Educational Use Only

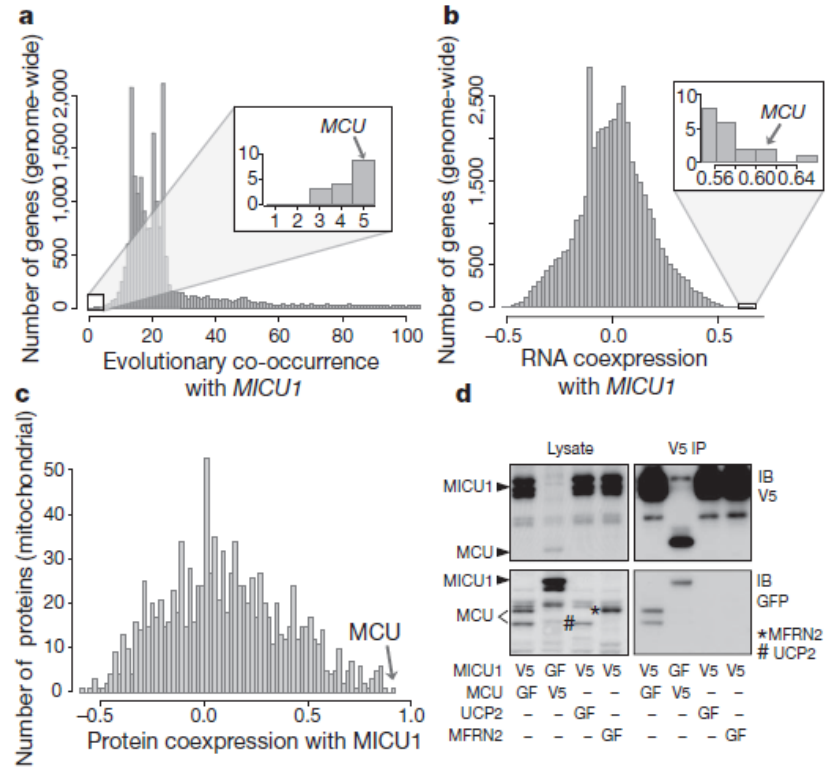


dimer

via PyMol



# The main movement: The discovery of MCU by Mootha VK. Group



整合基因组分析预测MCU与MICU1存在功能上的相关性

Nature. 2011 Jun 19;476(7360):341-5.



# How to open the scientific secret buried in the half century: The strategy of Rizzuto R. Group

The **Rizzuto R. Group** expect and made bioinformatics analysis step by step:

- (1) ubiquitous expression in mammalian tissues-- **529 candidates**
- (2) two or more predicted transmembrane domains-- **89 proteins**
- (3) absent in *Saccharomyces cerevisiae*-- 20
- (4) significantly conserved also in kinetoplastids-- **14**

This list included **13 proteins** with either already characterized functions (complex I ubunits, assembly regulators, and so on) or poor alignment scores.

Accession	Description
NP_062788	HIG1 domain family member 1A
NP_079736	transmembrane protein 126A
NP_510963	growth hormone-inducible transmembrane protein
NP_080638	transmembrane protein C9orf46 homolog
NP_079984	transmembrane protein 186
NP_904328	NADH dehydrogenase subunit 1
NP_904337	NADH dehydrogenase subunit 4
NP_904339	NADH dehydrogenase subunit 6
NP_081214	transmembrane protein 160
NP_780421	transmembrane protein 65
NP_081406	AFG3-like protein 2
XP_001478993	PREDICTED: NADH-ubiquinone oxidoreductase chain 5-like
NP_001028431	coiled-coil domain-containing protein 109A
NP_077235	transmembrane protein C3orf1 homolog

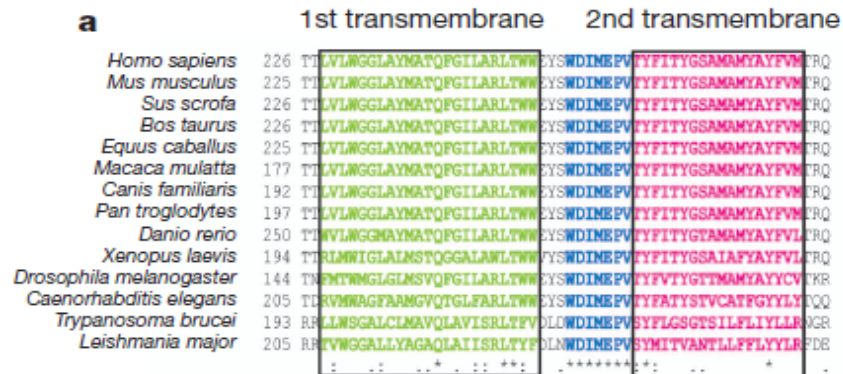


# The main movement: The discovery of MCU by Rizzuto R. Group

The remaining protein (NP\_001028431) includes

- 1 a highly conserved domain comprising two transmembrane regions
- 2 an intervening loop enriched in acidic residues, indicative of a possible common role in cation permeation.
- 3 This domain was also detected by hidden Markov model-based analysis of evolutionary conserved proteins (Pfam PF04678 and Panther PTHR1346 domains, with undefined function), further supporting the significance of the hit.

We thus considered this protein a putative mitochondrial Ca<sup>2+</sup> channel, named it MCU(mitochondrial calcium uniporter).





# MCU: Amino acids sequence and distribution

Residue	Number	Mole%
A = Ala	27	7.692
B = Asx	0	0
C = Cys	5	1.425
D = Asp	20	5.698
E = Glu	20	5.698
F = Phe	10	2.849
G = Gly	25	7.123
H = His	9	2.564
I = Ile	16	4.558
J = ---	0	0
K = Lys	16	4.558
L = Leu	45	12.821
M = Met	7	1.994
N = Asn	7	1.994
O = ---	0	0
P = Pro	13	3.704
Q = Gln	21	5.983
R = Arg	29	8.262
S = Ser	18	5.128
T = Thr	18	5.128
U = ---	0	0
V = Val	25	7.123
W = Trp	5	1.425
X = Xaa	0	0
Y = Tyr	15	4.274
Z = Glx	0	0

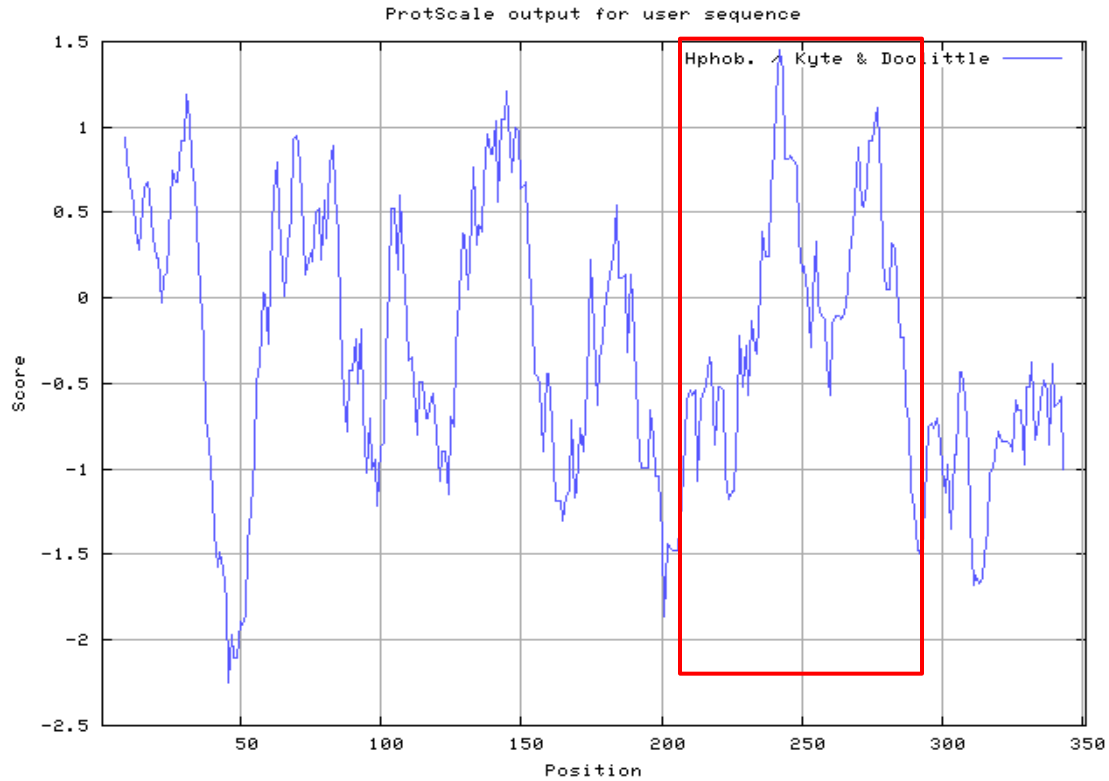
Property	Residues	Number	Mole%
Tiny	(A+C+G+S+T)	93	26.496
Small	(A+B+C+D+G+N+P+S+T+V)	158	45.014
Aliphatic	(A+I+L+V)	113	32.194
Aromatic	(F+H+W+Y)	39	11.111
Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	193	54.986
Polar	(D+E+H+K+N+Q+R+S+T+Z)	158	45.014
Charged	(B+D+E+H+K+R+Z)	94	26.781
Basic	(H+K+R)	54	15.385
Acidic	(B+D+E+Z)	40	11.396

Web lab--pepstats





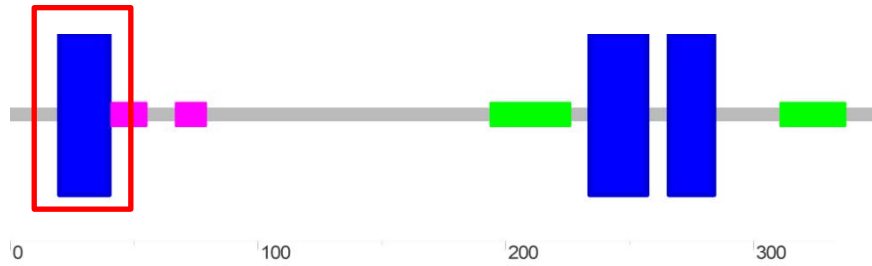
# MCU: Hydrophobic and hydrophilic Amino acids



ExPASy—ProtScale--Hphob. / Kyte & Doolittle

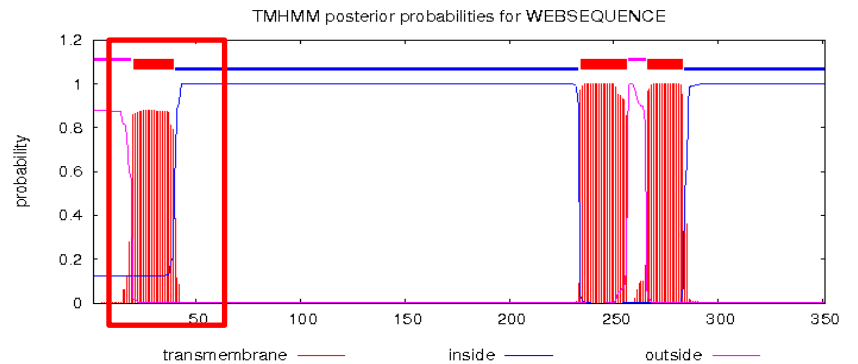


# MCU: Transmembrane prediction



Smart

TMHMM



## PTM / Processing<sup>i</sup>

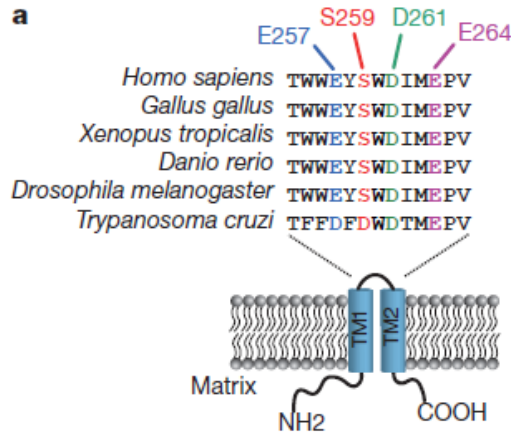
### Molecule processing

Uniprot

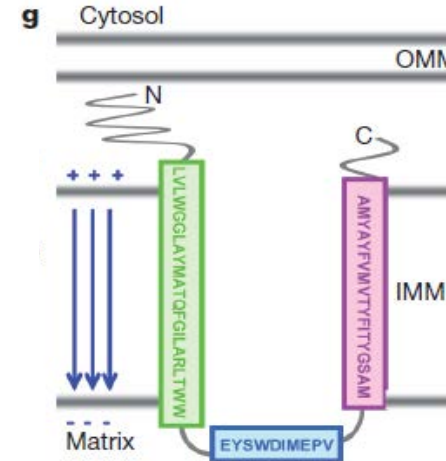
Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Transit peptide <sup>i</sup>	1 - 50	50	Mitochondrion <a href="#">Sequence Analysis</a>			<a href="#">Add</a> <a href="#">BLAST</a>
Chain <sup>i</sup>	51 - 351	301	Calcium uniporter protein, mitochondrial		PRO_0000282976	<a href="#">Add</a> <a href="#">BLAST</a>



# MCU: the direction of N- and C-terminal



VS



Nature. 2011 Jun 19;476(7360):336-40.

Nature. 2011 Jun 19;476(7360):341-5.

## Subcellular location<sup>1</sup>

Mitochondrion inner membrane 3 Publications ; Multi-pass membrane protein 3 Publications

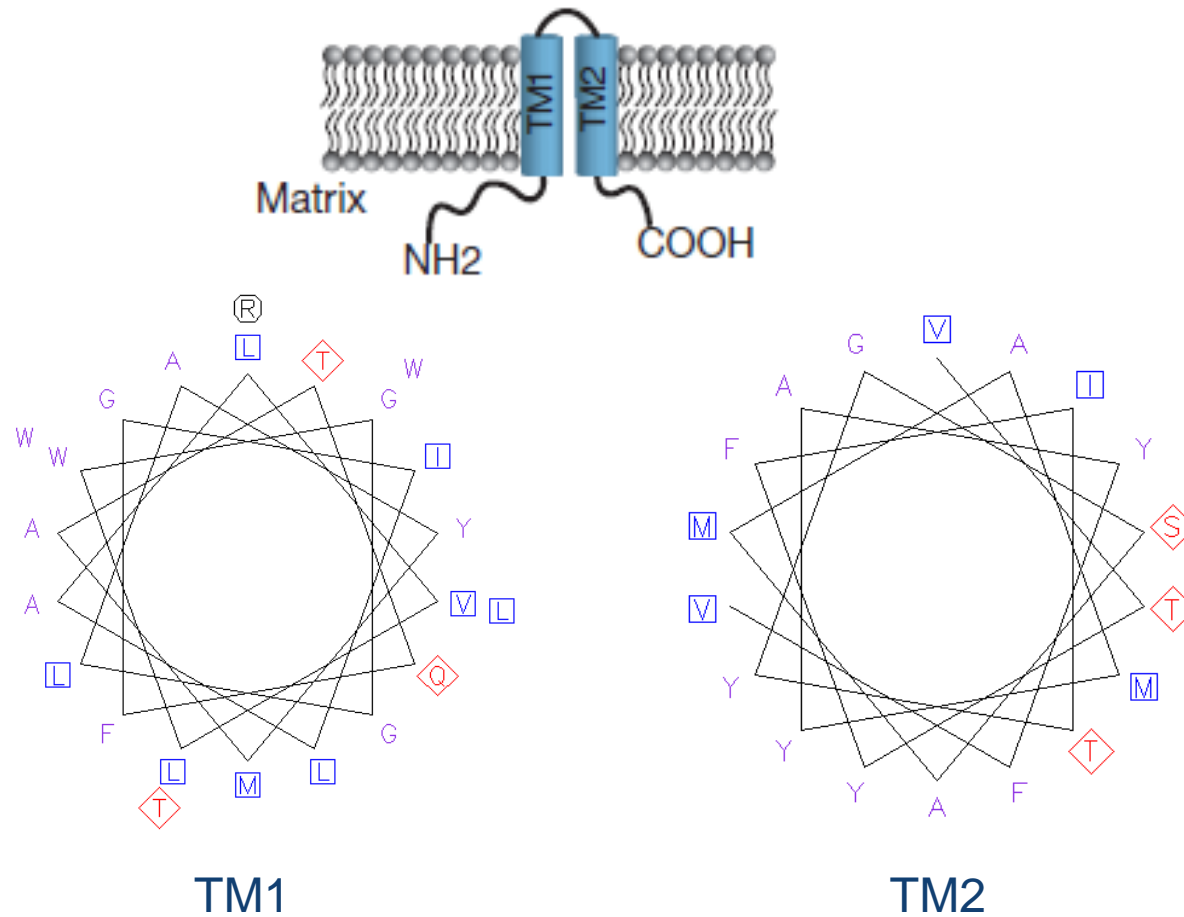
## Topology

Feature key	Position(s)	Length	Description	Graphical view	Feature identifier	Actions
Topological domain <sup>1</sup>	51 - 233	183	Mitochondrial matrix  Sequence Analysis			Add BLAST
Transmembrane <sup>1</sup>	234 - 256	23	Helical  Sequence Analysis			Add BLAST
Topological domain <sup>1</sup>	257 - 265	9	Mitochondrial intermembrane  Sequence Analysis			
Transmembrane <sup>1</sup>	266 - 283	18	Helical  Sequence Analysis			Add BLAST
Topological domain <sup>1</sup>	284 - 351	68	Mitochondrial matrix  Sequence Analysis			Add BLAST

Uniprot



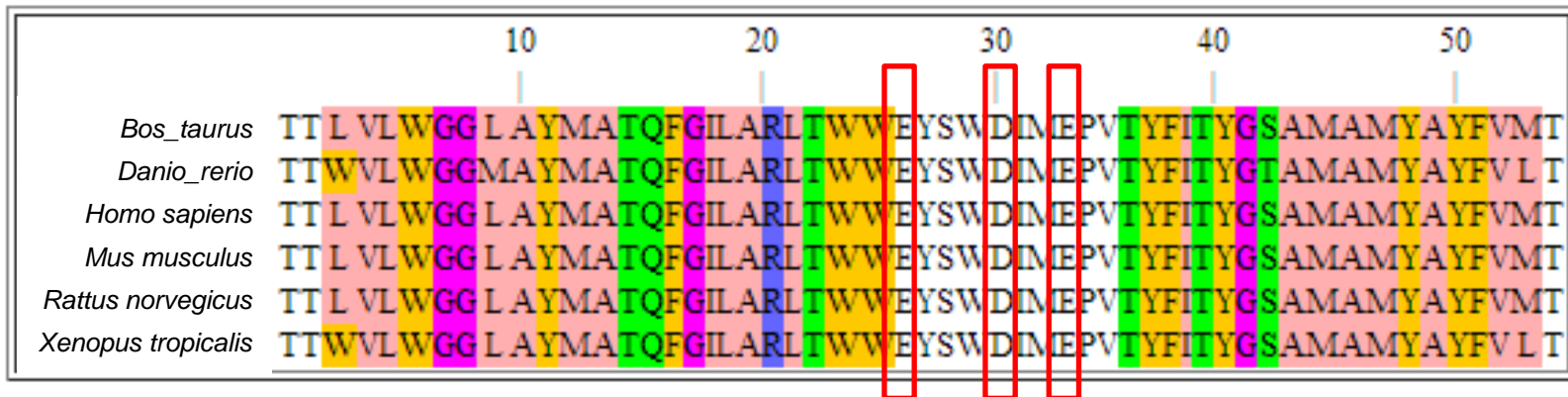
# MCU: transmembrane helix



Weblab-pepwheel



## MCU: Cation permeation



Glu(E) and Asp(D)-- **Acidic residues**—calcium permeation

Jalview



# MCU: coiled-coil domains and DUF607

Homo sapiens

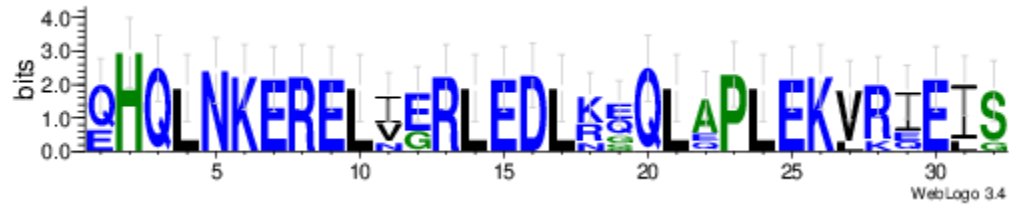
Mus musculus

Rattus norvegicus

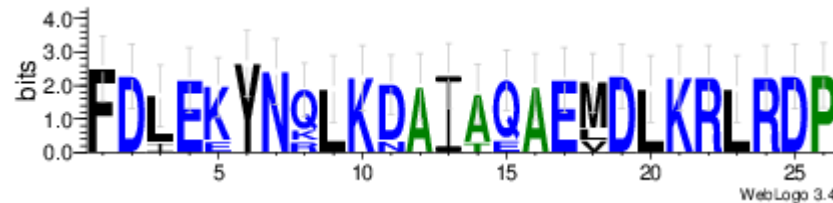
Bos taurus

Xenopus (Silurana) tropicalis

Danio rerio



Coiled coil 1



Coiled coil 2

Weblogo



Smart

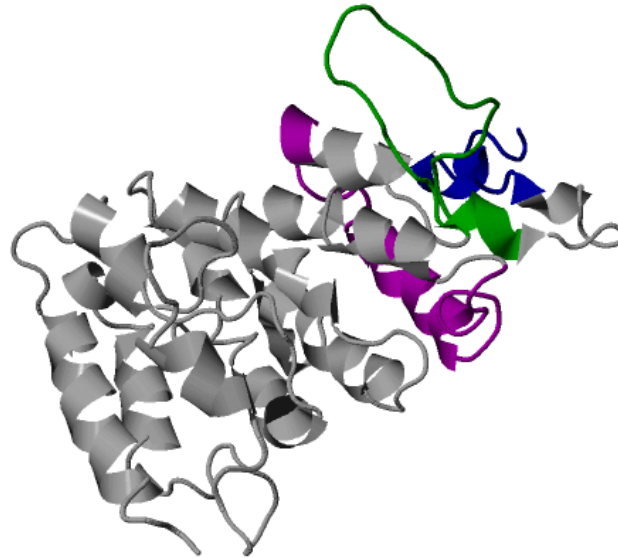
DUF607 superfamily represents a conserved region found in several uncharacterised eukaryotic proteins.



# MCU: Predicted 3D structure

ProteinModelPortal

ModBase



Jmol

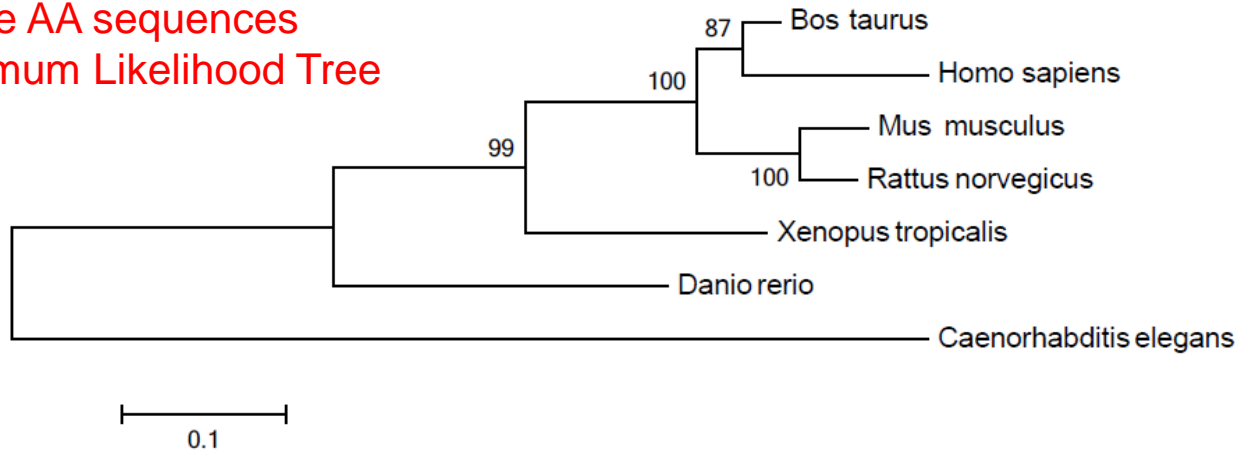
<input type="checkbox"/>	InterPro Domain	109 - 321	DUF607
<input checked="" type="checkbox"/>	Transmembrane	234 - 256	Helical; (Potential).
<input checked="" type="checkbox"/>	Transmembrane	266 - 283	Helical; (Potential).
<input checked="" type="checkbox"/>	Coiled coil	192 - 223	Potential.
<input type="checkbox"/>	Alternative sequence	166 - 219	DLLSHENAATLNDVKTLVQQLYTTLCIEQHQLNKERELIER



# MCU: Evolutional conservation analysis

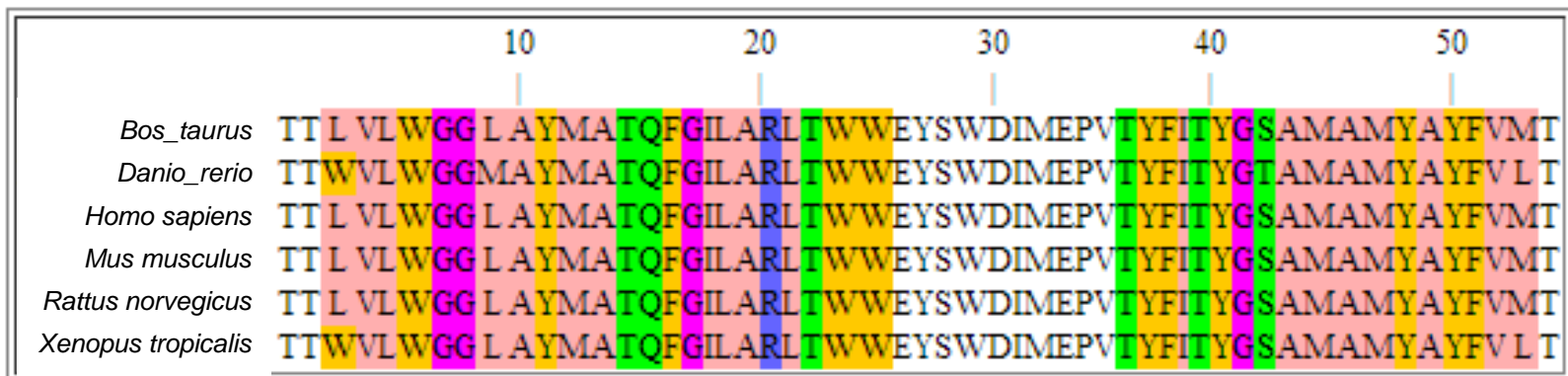
## MCU in seven species and their evolutionary conservations

Whole AA sequences  
Maximum Likelihood Tree



Mega 6.0

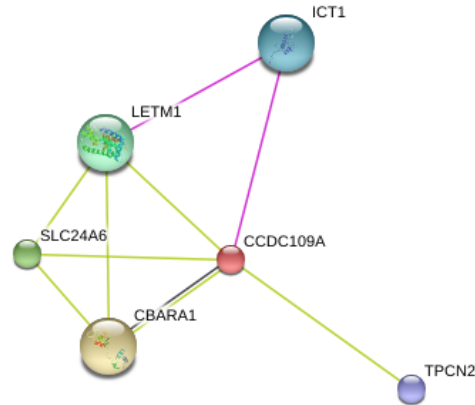
## Transmembrane domains and mitochondrial intermembrane







# MCU: Interaction with string



This is the **evidence view**. Different line colors represent the types of evidence for the association.

## Your Input:

- CCDC109A coiled-coil domain containing 109A (351 aa)  
(*Homo sapiens*)

## Predicted Functional Partners:

		Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
● CBARA1	calcium binding atopy-related autoantigen 1; Induces T helper 1-mediated autoreactivity, which [...]				●					0.683
● SLC24A6	solute carrier family 24 (sodium/potassium/calcium exchanger), member 6; Transports Ca(2+) in e [...]				●					0.540
● LETM1	leucine zipper-EF-hand containing transmembrane protein 1; Crucial for the maintenance of mitoc [...]									0.466
● ICT1	immature colon carcinoma transcript 1 (206 aa)							●		0.417
● TPCN2	two pore segment channel 2; May function as one of the major voltage-gated Ca(2+) channel (VDCC [...])								●	0.409

STRING



## Summary and Lessons

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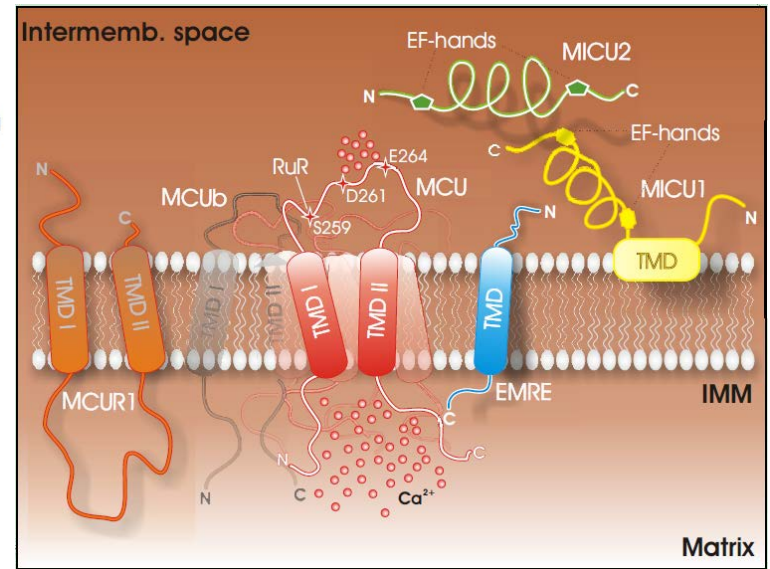
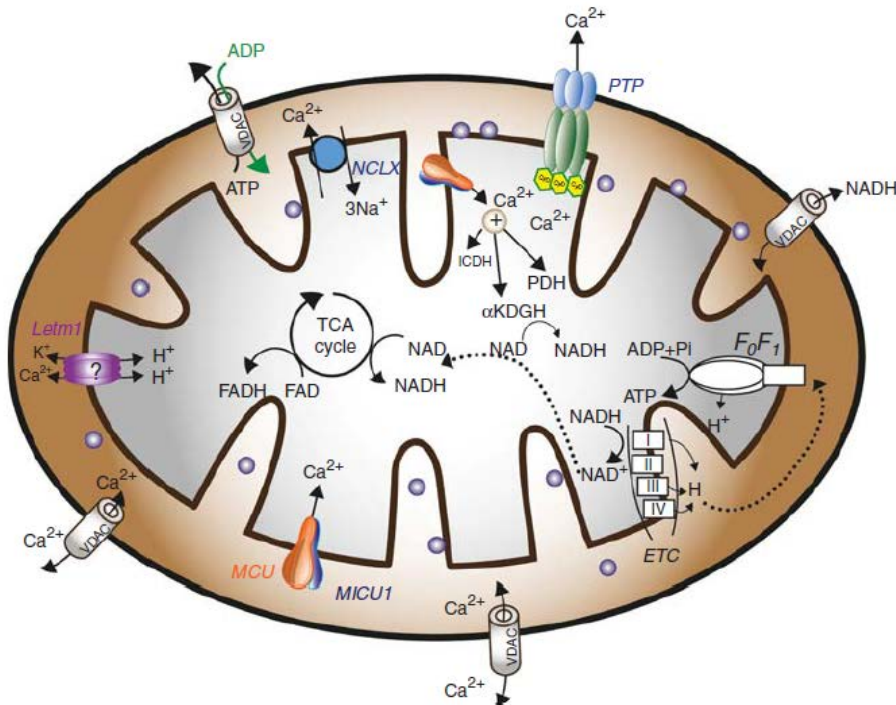
1 Bioinformatics had a significant role in the discovery of mitochondrial calcium uniporter.

2 Bioinformatics could provide us several insightful and efficient clues during the processes of searching for novel proteins.

3 For the proteins newly found, bioinformatics will help us to study the functions of novel proteins.



# Perspective



Cold Spring Harb Protoc. 2014 Feb 1;2014(2):161-6.

J Physiol. 2014 Mar 1;592(Pt 5):829-39.



**Thank You !**

