RESEARCH REVIEW JOURNALS
Volume-03
Issue-03
March-2018

ISSN: 2455-3085 (Online)
RESEARCH REVIEW International Journal of Multidisciplinary
www.rrjournals.com [UGC Listed Journal]

Insilico insights in the Functional characterizationof Calreticulin Protein

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ARTICLE DETAILS

Article History

Published Online: 30 March 2018

Keywords
Calreticulin, Chaperone, physicochemical properties,*insilico*,protein interactions.

ABSTRACT

Calreticulin is a multifunctional protein that acts as a major Ca(2+)-binding protein in the lumen of the Endoplasmic reticulum. It is a small size protein. It is seen in vertebrates, invertebrates, and higher plants. It assists in protein folding and acts as Ca2+ buffer inside sarcomeres and outside the Endoplasmic Reticulum in many functional and pathological responses. It is remarked that Calreticulin over-expression or its absence is associated with pathological conditions, such as cancer progression. An insilico attempt has been made to study the functional aspects of Calreticulin protein. Various online Bioinformatics tools were used to characterize and predict the physicochemical properties and protein-protein interactions of Calreticulin protein.

INTRODUCTION

Calreticulin was regarded as Ca2+-binding protein in the sarcoplasmic reticulum (SR) of skeletal muscle [1]. Its other aliases areCalregulin, CRP55, CaBP3,ERp60. In humans it is encoded by the Calreticulin gene. It is a multifunctional protein that acts as a major Ca 2+ binding protein in the lumen of the endoplasmic reticulum i.e. maintaining adequate calcium levels in organisms.[2]

Calreticulin is localized in Endoplasmic reticulum and inthe nucleus, suggesting its role in transcription regulation. Calreticulin successfullybinds to the synthetic peptide KLGFFKR, which is similar to the amino acid sequence in the DNA-binding domain of the largest superfamily of nuclear receptors[3].

The N- terminalregion of Calreticulin protein interacts with the DNA-binding domain of the Glucocorticoid receptor and inhibits the receptor from binding to its specificresponse element. Calreticulin has a significant role in the regulation of transcription by nuclear hormone receptors [3,27]. It also plays a key role in processes like wound healing, cancer cell removal, and parasite development.

FUNCTIONS OF CALRETICULIN

Calreticulin hascontributedto various physiological and pathological processes in the cells. The non-ER Calreticulin also controls various biological functions like cell adhesion, formation of functional gene product and exerting control over RNA stability.

1. Calcium Homeostasis

Ca2+ is a widespread signaling molecule involved in the development of the organism and other cellular processes [4]. Ca2+-binding chaperones influence Ca2+ storage in the lumen of ER[5-8].Calreticulin is a Ca2+ regulator as it has two Ca2+-binding sites in the P-domain and C-domain. More than half of Ca2+ stored in the lumen of ER shows binding affinity with Calreticulin. Thus, increased levels of Calreticulin may lead to increase in intracellular Ca2+ storage[9]. In contrast, Calreticulin-deficient cells have a low Ca2+ storage in the lumen of ER.

2. Cell Adhesion

It has been studied that any modification in the Calreticulin levels affects cell adhesion in theextracellular matrix [10,11]. Calreticulin influences the cell adhesionby regulating fibronectin gene expression and its rate of deposition in the matrix. Calreticulin-mediated cell adhesion is due to associationof Calreticulin and integrins by binding to the cytoplasmic motif of the α-subunit of Integrin protein [12].

3. RNA Stability

Calreticulin is a mRNA binding protein that disrupts angiotensin receptor by binding to AU-rich region in 3′-UTR[13]. Calreticulin also binds with glucose transporter-1 mRNA and destabilizes the mRNA under high-glucose conditions [14]. Thus, Calreticulin also acts regulates mRNA stability.

4. Regulation of Calreticulin Expression

The human Calreticulin gene has nine exons and is located on chromosome 19. There are several binding sites for transcription factors on the promoter of Calreticulin gene which act as modulators of Calreticulin expression. Loss of Calcium deposits and ER stress are significant activators of Calreticulin expression [15,16]. Nerve growth factor (NGF)also enhancesCalreticulin mRNA expression in ovarian cells anddifferentiation of neurons [17]. Thus, Calreticulin has a rolein biological and pathological processes.

5. Clinical Impacts of Calreticulin Expression in Different Cancers

Many studies have shown relationship between Calreticulin expression levels in different cancerous tissues showing that tumor tissues express higher levels of Calreticulin as compared to wild type tissues[18].

6.As a Phagocytic Signal

Calreticulin induces immune response which is significant for the destroying cancerous cells [19,20]. Calreticulin aids in the phagocytic uptake of apoptotic and cancer cells [21]. Clarke and Smyth examined that specific drugs exposed a surface prophagocytic protein, Calreticulin, which stimulated immunogenic cell death [22].

Genomic view of Calreticulin Gene

Calreticulin has a total of 19 Chromosomes that start at 12,938,578 bp and end at 12,944,490 bp with size of 5,913bases and Orientation on Plus strand as illustrated in the NCBI Genome Database View (Fig 2)

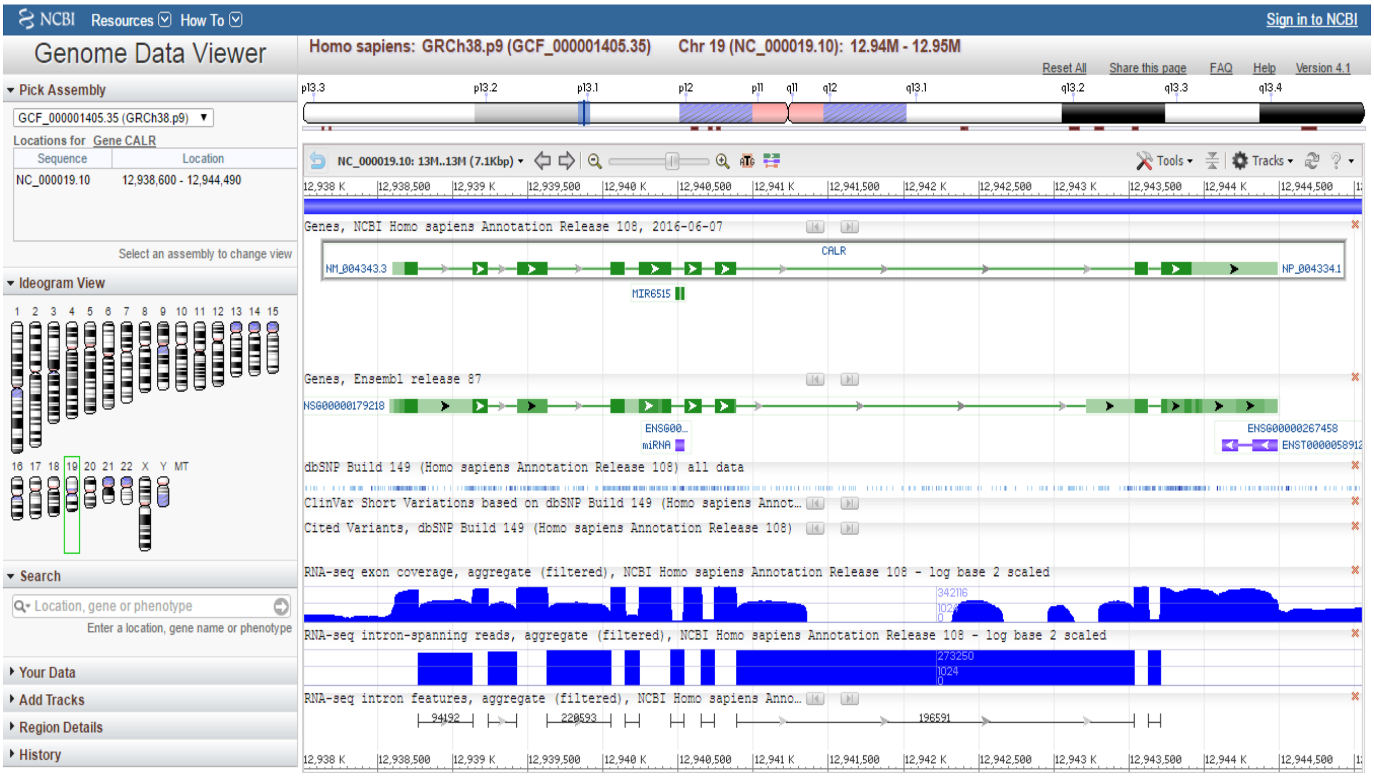


Fig 2: Genome Data View of Calreticulin gene

Source: https://www.ncbi.nlm.nih.gov/genome/gdv/?context=gene&acc=811

METHODOLOGY

Sequence Retrieval: Calreticulin protein sequence in *Homo sapiens* (Accession number- **AAB51176**) was retrieved from NCBI protein database having length of 417 residues. (Fig 4)

Subcellular Localization of Calreticulin using PSORT II- (Url: https://psort.hgc.jp/)

PSort II is a computer program for the probable identification of protein localization sites in cells. It accepts the details of an amino acid sequence and its source. It computes the given sequence for the features of the previously known protein signals. Finally, it gives the likelihood for the input protein to be situated at each candidate site with additional information. (Fig 5)

Amino acid scale using ProtScale (Url: http://web.expasy.org/cgi-bin/protscale/protscale.pl)

ProtScale computes the profile produced by amino acid scale on a selected protein (Fig 6). It accepts SwissProt/TrEMBL accession number or sequence in desired format. By default, it considers, Hydrophobicity/ Kyte& Doolittle amino acid scale which displays information related to the author, reference, amino acid scale values.

Protein-protein interactions using STRING Database (Url: http://string-db.org/)

STRING database gives the information about protein interactions. The interactions include the physical and functional relationships. They are derived from various sources like Genomic context, high throughput experiments, co-expression, previous knowledge (fig 8).

Conserved domains analysis using CDD(Url:http://www.ncbi.nlm.nih.gov/cdd/)

The conserved domain database is used to annotate the functional units in proteins. It is a collection of Domain models curated by NCBI, which utilizes 3D structure to give insights in the sequence, structure and function relationships.

Functional annotations using Uniprot (Url: http://uniprot.org/uniprot/)

Uniprot is a complete resource for protein sequence and annotation information.

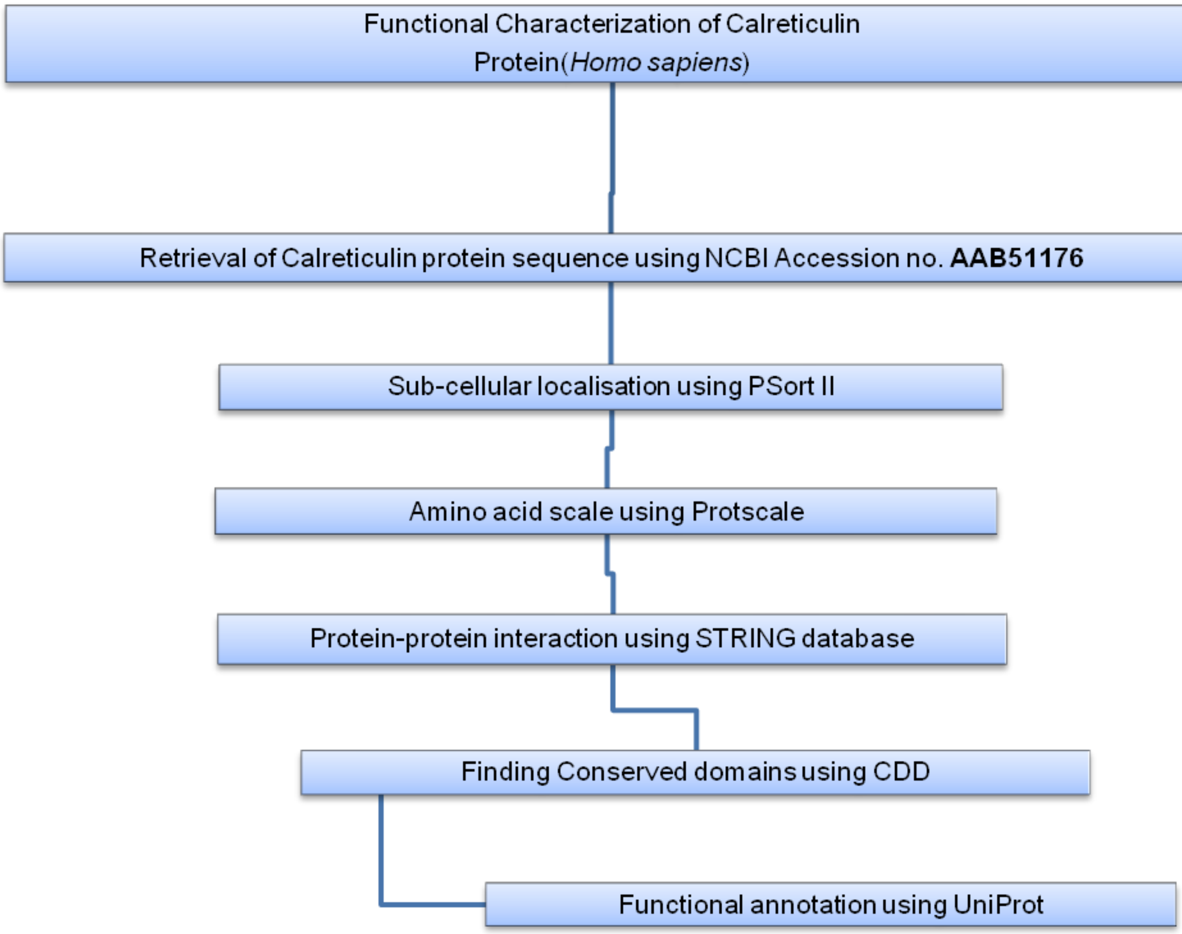
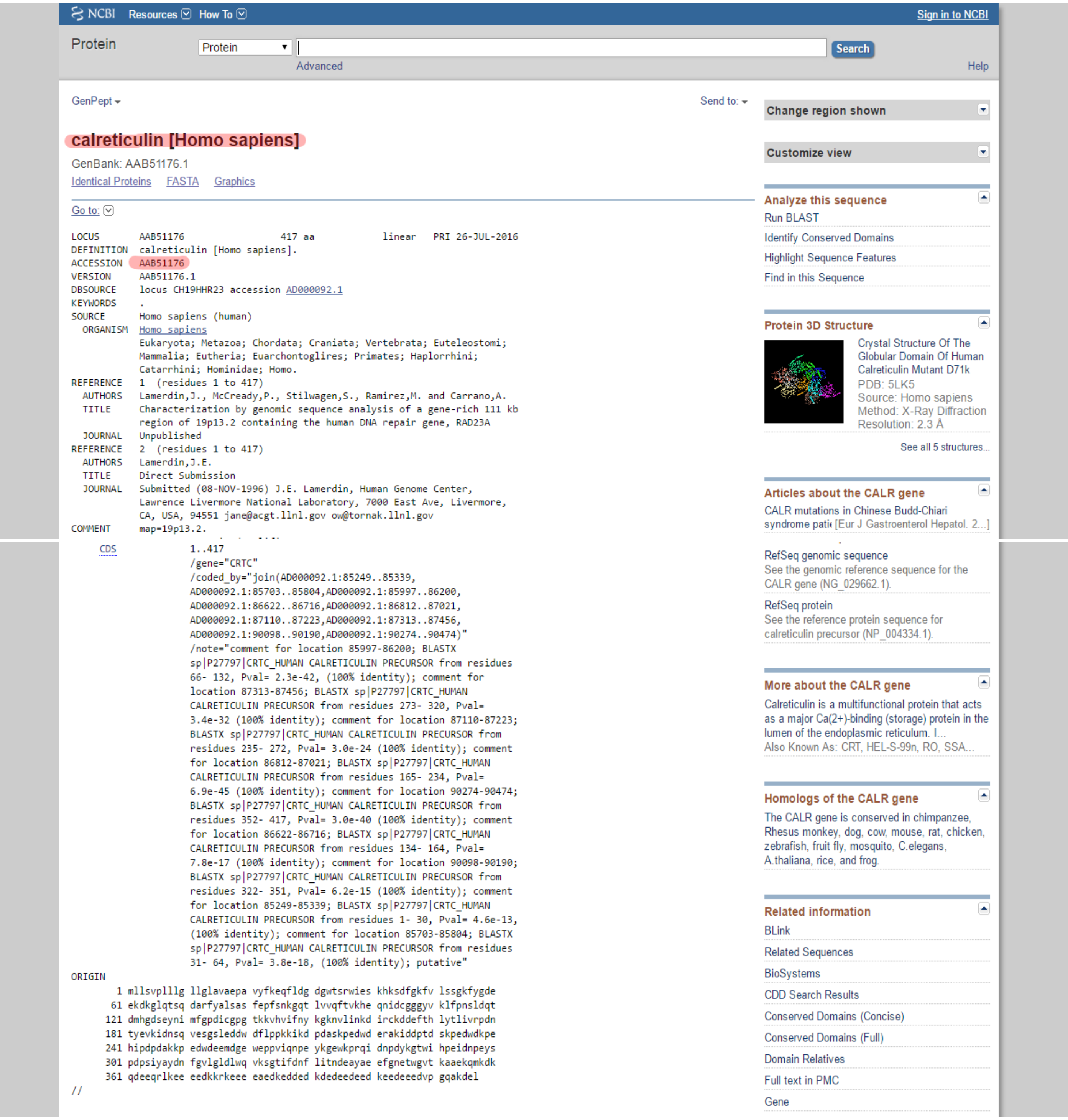


Figure:3 Strategy used for the Characterizing Calreticulin Protein

RESULTS AND DISCUSSIONS:

The Calreticulin protein was retrieved from GenPept database, NCBI.



calreticulin [Homo sapiens]

GenBank: AAB51176.1

Identical Proteins FASTA Graphics

```
LOCUS       AAB51176                 417 aa            linear   PRI 26-JUL-2016
DEFINITION  calreticulin [Homo sapiens].
ACCESSION   AAB51176
VERSION     AAB51176.1
DBSOURCE    locus CH19HHR23 accession AD000092.1
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 417)
  AUTHORS   Lamerdin,J., McCready,P., Stilwagen,S., Ramirez,M. and Carrano,A.
  TITLE     Characterization by genomic sequence analysis of a gene-rich 111 kb
            region of 19p13.2 containing the human DNA repair gene, RAD23A
  JOURNAL   Unpublished
REFERENCE   2  (residues 1 to 417)
  AUTHORS   Lamerdin,J.E.
  TITLE     Direct Submission
  JOURNAL   Submitted (08-NOV-1996) J.E. Lamerdin, Human Genome Center,
            Lawrence Livermore National Laboratory, 7000 East Ave, Livermore,
            CA, USA, 94551 jane@acgt.llnl.gov ow@tornak.llnl.gov
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                     AD000092.1:85703..85804,AD000092.1:85997..86200,
                     AD000092.1:86622..86716,AD000092.1:86812..87021,
                     AD000092.1:87110..87223,AD000092.1:87313..87456,
                     AD000092.1:90098..90190,AD000092.1:90274..90474)"
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                     location 87313-87456; BLASTX sp|P27797|CRTC_HUMAN
                     CALRETICULIN PRECURSOR from residues 273- 320, Pval=
                     3.4e-32 (100% identity); comment for location 87110-87223;
                     BLASTX sp|P27797|CRTC_HUMAN CALRETICULIN PRECURSOR from
                     residues 235- 272, Pval= 3.0e-24 (100% identity); comment
                     for location 86812-87021; BLASTX sp|P27797|CRTC_HUMAN
                     CALRETICULIN PRECURSOR from residues 165- 234, Pval=
                     6.9e-45 (100% identity); comment for location 90274-90474;
                     BLASTX sp|P27797|CRTC_HUMAN CALRETICULIN PRECURSOR from
                     residues 352- 417, Pval= 3.0e-40 (100% identity); comment
                     for location 86622-86716; BLASTX sp|P27797|CRTC_HUMAN
                     CALRETICULIN PRECURSOR from residues 134- 164, Pval=
                     7.8e-17 (100% identity); comment for location 90098-90190;
                     BLASTX sp|P27797|CRTC_HUMAN CALRETICULIN PRECURSOR from
                     residues 322- 351, Pval= 6.2e-15 (100% identity); comment
                     for location 85249-85339; BLASTX sp|P27797|CRTC_HUMAN
                     CALRETICULIN PRECURSOR from residues 1- 30, Pval= 4.6e-13,
                     (100% identity); comment for location 85703-85804; BLASTX
                     sp|P27797|CRTC_HUMAN CALRETICULIN PRECURSOR from residues
                     31- 64, Pval= 3.8e-18, (100% identity); putative"
ORIGIN
        1 mllsvplllg llglavaepa vyfkeqfldg dgwtsrwies khksdfgkfv lssgkfygde
       61 ekdkglqtsq darfyalsas fepfsnkgqt lvvqftvkhe qnidcgggyv klfpnsldqt
      121 dmhgdseyni mfgpdicgpg tkkvhvifny kgknvlinkd irckddefth lytlivrpdn
      181 tyevkidnsq vesgsleddw dflppkkikd pdaskpedwd erakiddptd skpedwdkpe
      241 hipdpdakkp edwdeemdge weppviqnpe ykgewkprqi dnpdykgtwi hpeidnpeys
      301 pdpsiyaydn fgvlgldlwq vksgtifdnf litndeayae efgnetwgvt kaaekqmkdk
      361 qdeeqrlkee eedkkrkeee eaedkedded kdedeedeed keedeeedvp gqakdel
//
```

Analyze this sequence

Run BLAST

Identify Conserved Domains

Highlight Sequence Features

Find in this Sequence

Protein 3D Structure

Crystal Structure Of The Globular Domain Of Human Calreticulin Mutant D71k
PDB: 5LK5
Source: Homo sapiens
Method: X-Ray Diffraction
Resolution: 2.3 Å

See all 5 structures...

Articles about the CALR gene

CALR mutations in Chinese Budd-Chiari syndrome pati‹ [Eur J Gastroenterol Hepatol. 2...]

RefSeq genomic sequence
See the genomic reference sequence for the CALR gene (NG_029662.1).

RefSeq protein
See the reference protein sequence for calreticulin precursor (NP_004334.1).

More about the CALR gene

Calreticulin is a multifunctional protein that acts as a major Ca(2+)-binding (storage) protein in the lumen of the endoplasmic reticulum. I...
Also Known As: CRT, HEL-S-99n, RO, SSA...

Homologs of the CALR gene

The CALR gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, chicken, zebrafish, fruit fly, mosquito, C.elegans, A.thaliana, rice, and frog.

Related information

BLink

Related Sequences

BioSystems

CDD Search Results

Conserved Domains (Concise)

Conserved Domains (Full)

Domain Relatives

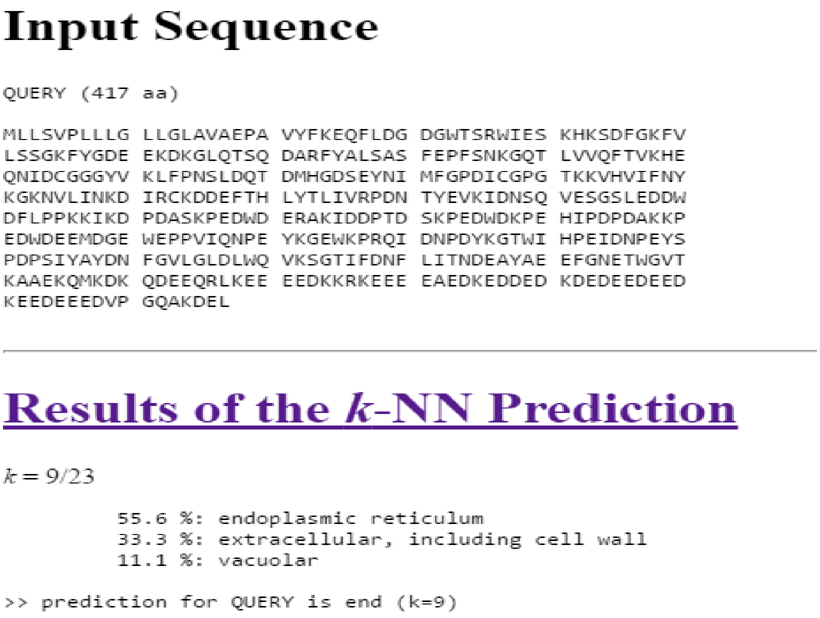
Full text in PMC

Gene

Fig4: Calreticulin [*Homo sapiens*] protein retrieved inGenpept format

The sub-cellular localization as predicted by PSORTIIrevealed that Calreticulin islocalized in endoplasmic reticulum(55.6%), extracellular region (33.3 %) including cell wall and 11.1 % in vacuolar sites (Fig 5)

The ProtScale analysis illustrated that Calregulin is highly hydrophilic due to high content of polar residues approximately 66.2%. It demonstrates that the GRAVITY index is negative, indicating that Calreticulin interacts more efficiently with water, like a protein of hydrophilic nature (Fig 6a and b).



Input Sequence

```
QUERY (417 aa)

MLLSVPLLLG LLGLAVAEPA VYFKEQFLDG DGWTSRWIES KHKSDFGKFV
LSSGKFYGDE EKDKGLQTSQ DARFYALSAS FEPFSNKGQT LVVQFTVKHE
QNIDCGGGYV KLFPNSLDQT DMHGDSEYNI MFGPDICGPG TKKVHVIFNY
KGKNVLINKD IRCKDDEFTH LYTLIVRPDN TYEVKIDNSQ VESGSLEDDW
DFLPPKKIKD PDASKPEDWD ERAKIDDPTD SKPEDWDKPE HIPDPDAKKP
EDWDEEMDGE WEPPVIQNPE YKGEWKPRQI DNPDYKGTWI HPEIDNPEYS
PDPSIYAYDN FGVLGLDLWQ VKSGTIFDNF LITNDEAYAE EFGNETWGVT
KAAEKQMKDK QDEEQRLKEE EEDKKRKEEE EAEDKEDDED KDEDEEDEED
KEEDEEEDVP GQAKDEL
```

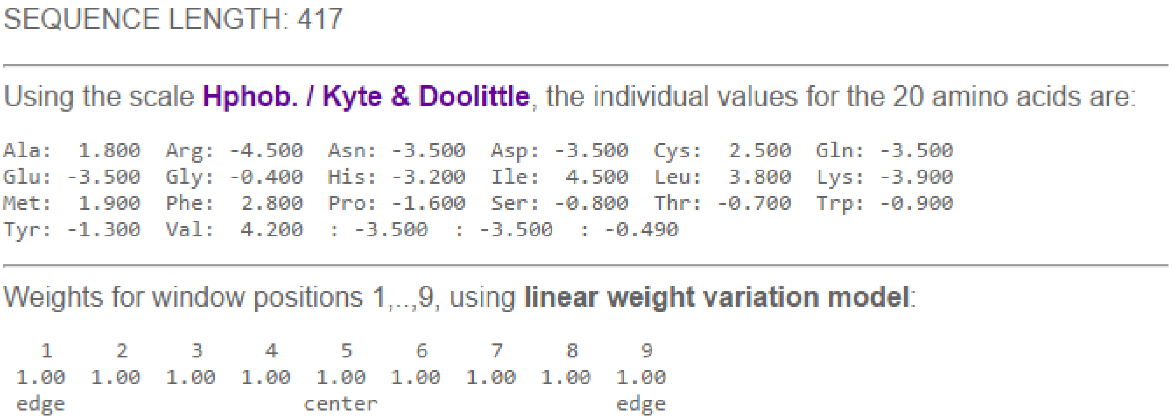
Results of the *k*-NN Prediction

k = 9/23

```
        55.6 %: endoplasmic reticulum
        33.3 %: extracellular, including cell wall
        11.1 %: vacuolar

>> prediction for QUERY is end (k=9)
```

Fig 5: Sub-cellular localization of Calreticulin protein using PSORT II



SEQUENCE LENGTH: 417

Using the scale **Hphob. / Kyte & Doolittle**, the individual values for the 20 amino acids are:

```
Ala:  1.800  Arg: -4.500  Asn: -3.500  Asp: -3.500  Cys:  2.500  Gln: -3.500
Glu: -3.500  Gly: -0.400  His: -3.200  Ile:  4.500  Leu:  3.800  Lys: -3.900
Met:  1.900  Phe:  2.800  Pro: -1.600  Ser: -0.800  Thr: -0.700  Trp: -0.900
Tyr: -1.300  Val:  4.200   : -3.500    : -3.500    : -0.490
```

Weights for window positions 1,..,9, using **linear weight variation model**:

```
   1     2     3     4     5     6     7     8     9
 1.00  1.00  1.00  1.00  1.00  1.00  1.00  1.00  1.00
 edge                    center                  edge
```

Fig 6(a) Kyte and Doolittle scale showing the individual values for Calreticulin protein

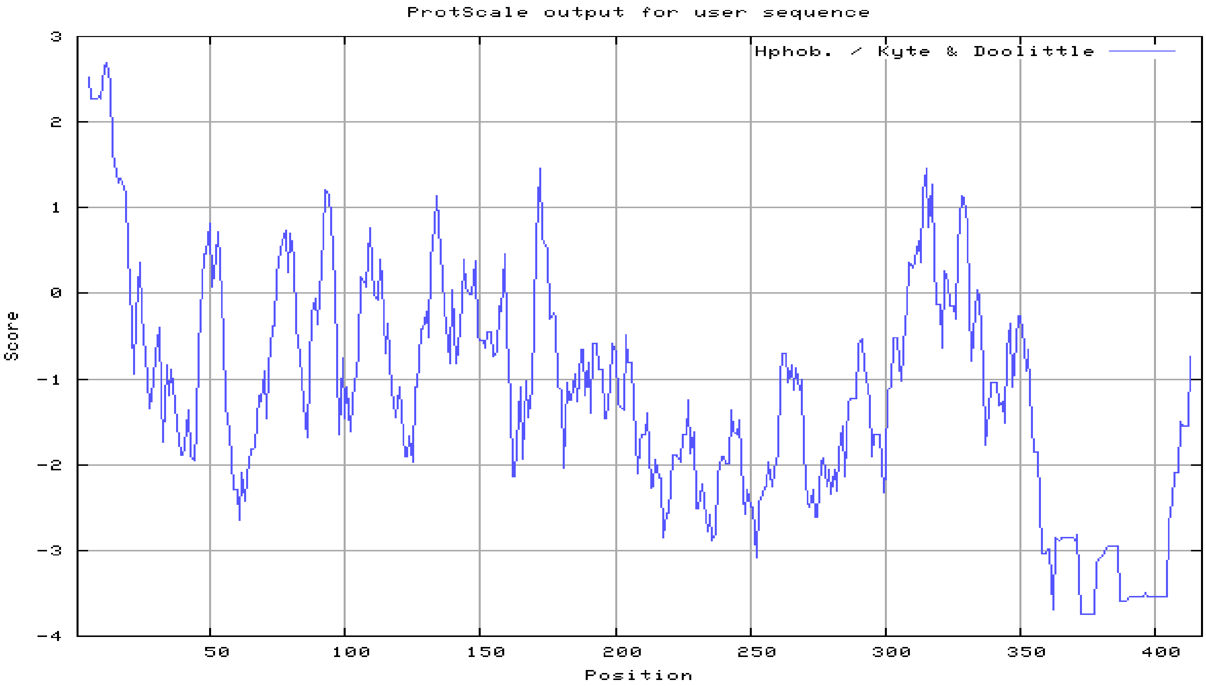
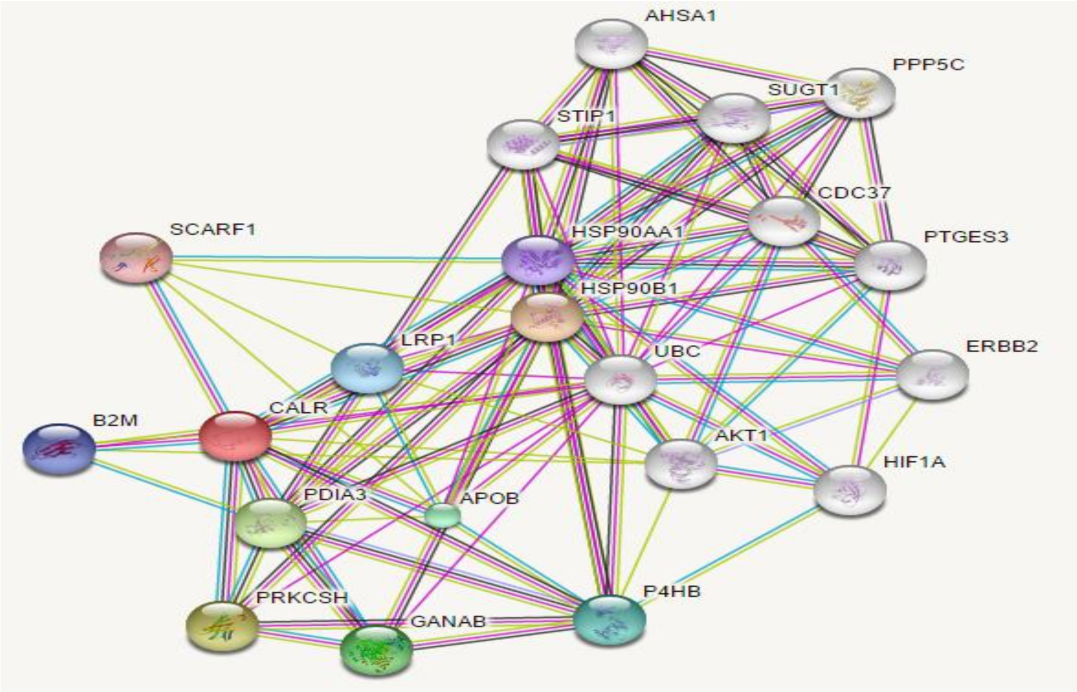
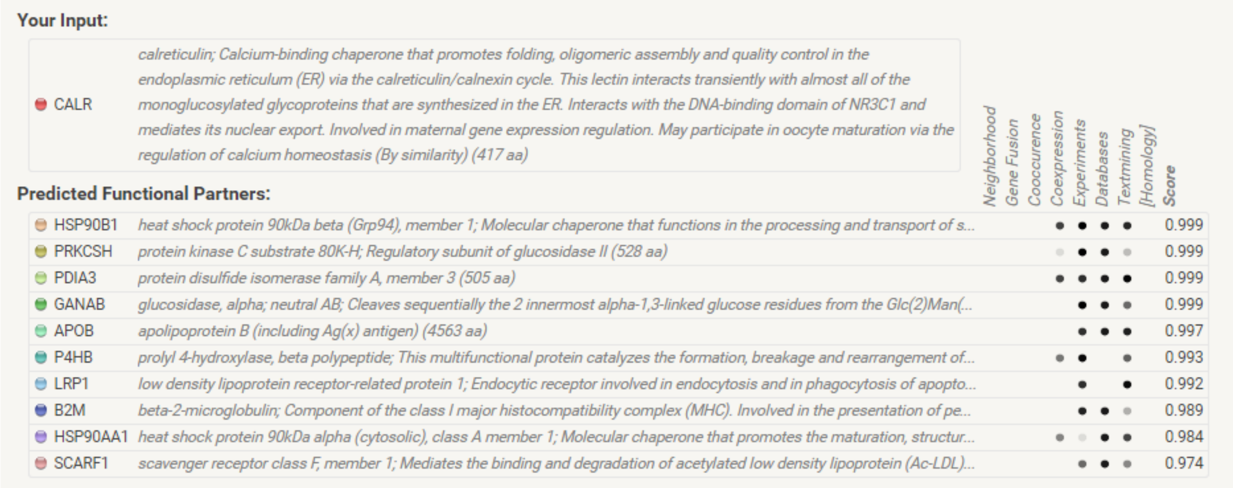


Fig 6 (b):Calreticulin with high hydrophilicity due to high content of polar regions using ProtScale

Some of the predicted functional interaction partners of Calreticulin protein in Humansas shown by STRING database are heat shock protein 90kDa beta, protein kinase C substrate 80K-H, protein disulfide isomerase family A, glucosidase(alpha) and apolipoprotein B etc.

The proteins are identified by their gene names located near each sphere. PPIs with score greater than 0.787 were selected to construct PPI networks. (Fig 7)





Your Input:

● CALR	calreticulin; Calcium-binding chaperone that promotes folding, oligomeric assembly and quality control in the endoplasmic reticulum (ER) via the calreticulin/calnexin cycle. This lectin interacts transiently with almost all of the monoglucosylated glycoproteins that are synthesized in the ER. Interacts with the DNA-binding domain of NR3C1 and mediates its nuclear export. Involved in maternal gene expression regulation. May participate in oocyte maturation via the regulation of calcium homeostasis (By similarity) (417 aa)	

Predicted Functional Partners:

		Neighborhood	Gene Fusion	Cooccurence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
● HSP90B1	heat shock protein 90kDa beta (Grp94), member 1; Molecular chaperone that functions in the processing and transport of s...				●	●	●	●		0.999
● PRKCSH	protein kinase C substrate 80K-H; Regulatory subunit of glucosidase II (528 aa)				●	●	●	●		0.999
● PDIA3	protein disulfide isomerase family A, member 3 (505 aa)				●	●	●	●		0.999
● GANAB	glucosidase, alpha; neutral AB; Cleaves sequentially the 2 innermost alpha-1,3-linked glucose residues from the Glc(2)Man(...					●	●	●		0.999
● APOB	apolipoprotein B (including Ag(x) antigen) (4563 aa)					●	●	●		0.997
● P4HB	prolyl 4-hydroxylase, beta polypeptide; This multifunctional protein catalyzes the formation, breakage and rearrangement of...				●	●		●		0.993
● LRP1	low density lipoprotein receptor-related protein 1; Endocytic receptor involved in endocytosis and in phagocytosis of apopto...					●		●		0.992
● B2M	beta-2-microglobulin; Component of the class I major histocompatibility complex (MHC). Involved in the presentation of pe...					●	●	●		0.989
● HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1; Molecular chaperone that promotes the maturation, structur...				●	●	●	●		0.984
● SCARF1	scavenger receptor class F, member 1; Mediates the binding and degradation of acetylated low density lipoprotein (Ac-LDL)...					●	●	●		0.974

Fig 7: Physical and functional associations of Calreticulin using STRING database

Uniprot was referred for functional annotations of Calreticulin to find N, P and C-domains. Uniprot results shows that N-domain exists at positions 18-197, P-domain at positions 198-308 and C-domain positioned at 309-416(in *Mus musculus*) and 309-417(*Homo sapiens*).

Region

Feature key	Position(s)	Description	Actions	Graphical view	Length
Region	18 – 197	N-domain	Add BLAST		180
Region	191 – 255	4 X approximate repeats	Add BLAST		65
Region	198 – 308	P-domain	Add BLAST		111
Region	237 – 270	Interaction with PPIB By similarity	Add BLAST		34
Region	259 – 297	3 X approximate repeats	Add BLAST		39
Region	309 – 417	C-domain	Add BLAST		109

a)

Region

Feature key	Position(s)	Description	Actions	Graphical view	Length
Region	18 – 197	N-domain	Add BLAST		180
Region	191 – 255	4 X approximate repeats	Add BLAST		65
Region	198 – 308	P-domain	Add BLAST		111
Region	237 – 270	Interaction with PPIB 1 Publication	Add BLAST		34
Region	259 – 297	3 X approximate repeats	Add BLAST		39
Region	309 – 416	C-domain	Add BLAST		108

b)

Fig 8: Functional annotations of Calreticulin using Uniprot in (a) Humans and (b) Mice

CONCLUSION

The present study emphasizes the functional characterization of Human Calreticulin protein using online computational tools. Calreticulin is associated with the Endoplasmic reticulum and is hydrophilic in nature with high content of polar residues (66.2%). Functional partners of Calreticulin as predicted by STRING having significant score are heat shock protein 90kDa beta, protein kinase C substrate 80K-H, protein disulfide isomerase family A, glucosidase(alpha) and apolipo protein B. Thus, the functional and physical networks of Calreticulin reveal that it interacts with DNA-binding domain and mediates nuclear export. It also interacts with mono-glycosylated proteins that are synthesized in ER and participates in exerting control of calcium metabolism.Uniprot annotations further clarified the occurrence of N, P and C domains in Humans and mice indicating that Calreticulin is conserved amongst them.

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