New Function of Calreticulin
Calreticulin-Dependent mRNA Destabilization
Mitsuhiro Yokoyama, Ken-ichi Hirata

Calreticulin was first identified as a Ca\(^{2+}\)-binding protein of the muscle sarcoplasmic reticulum in 1974, and the DNA encoding this protein was isolated in 1989. Calreticulin is a ubiquitous protein, found in a wide range of species and in all nucleated cell types, and has a variety of important biological functions. The human gene for calreticulin contains 9 exons and 8 introns. The deduced amino acid sequence indicates that calreticulin has a 17 amino acid hydrophobic signal sequence at its N terminus and that mature calreticulin contains 400 amino acids. The structure of calreticulin has been well characterized. It has at least 3 structural and functional domains (Figure).

Proposed functions for calreticulin range from chaperoning in the endoplasmic reticulum (ER) to antithrombotic effects at the cell surface, and from the regulation of Ca\(^{2+}\) signaling to the modulation of gene expression and cellular adhesion (Table). Two major functions of calreticulin in the ER lumen, ie, chaperoning and regulation of Ca\(^{2+}\) homeostasis, were intensively investigated and well characterized. Altered expression of calreticulin also has profound effects on many cellular functions. How can this one protein play a role in so many important cellular functions, and where within the cell does it carry out these functions? It is accepted that the majority of cellular calreticulin is located in the ER, where it plays important roles in molecular chaperoning and Ca\(^{2+}\) signaling. There is considerable evidence to indicate that calreticulin is found outside the ER, although how the protein relocates from the ER to the outside of the ER remains unclear. Functions of calreticulin outside the ER include modulation of cell adhesion, integrin-dependent Ca\(^{2+}\) signaling, and steroid-sensitive gene expression as well as mRNA destabilization both in vitro and in vivo. One major controversy in the calreticulin research field concerns the mechanisms involved in calreticulin-dependent modulation of functions outside the ER.

Destabilization of 3′-Untranslated Region of mRNA by Calreticulin
Angiotensin II plays a central role in cardiovascular homeostasis by regulating blood volume and vascular tone. In vascular smooth muscle cells, angiotensin II downregulates type 1 angiotensin II (AT\(_1\)) receptor, which appears to involve several mechanisms. AT\(_1\) receptor regulation takes place at the posttranscriptional level via agonist-induced destabilization of the AT\(_1\) receptor mRNA. Nickenting et al discovered that a novel mRNA binding protein, calreticulin, binds to the cognate sequence bases 2175 to 2195 within the 3′ untranslated region of the AT\(_1\) receptor mRNA. Angiotensin II stimulation, which causes destabilization of AT\(_1\) receptor mRNA, causes phosphorylation of calreticulin. This region comprises aAAUAAA hexamer and is considerably AU-rich.

Glucose transport in mammalian cells is mediated by a family of structurally related glycoproteins, the glucose transporters (GLUTs). GLUTs are usually expressed in a tissue-specific manner. In contrast, GLUT-1 is ubiquitous, being expressed in most cells, often together with other tissue-specific GLUTs such as GLUT-4 in vascular smooth muscle cells and endothelial cells. Transporter activity can be regulated by hormones, growth factors, and metabolites through translocation of the proteins, modulation of transporter intrinsic activity, and expression levels of the proteins. The expression of the GLUT-1 gene is principally regulated at the posttranscriptional level under a variety of pathophysiological conditions including glucose deprivation and hypoxia. The functional mapping of the GLUT-1 transcript identified several cis-acting regulatory elements that interact with trans-acting proteins to modulate the expression of this mRNA.

For example, adenosine-uridine (A-U)–binding protein, human heteronuclear ribonucleo-protein A\(_2\) (hn RNA A\(_2\)), and human embryonic lethal abnormal vision–like neuronal protein (Hel-N1) bind to U-rich regions of the GLUT-1 3′-untranslated region (UTR) to either increase or decrease the stability of this mRNA. There are at least 4 distinct cis-acting elements in GLUT-1 3′-UTR. An important 10 nucleotide cis-acting regulatory element (CAE) was localized within nucleotide 2181 to 8190 of the bovine GLUT-1 3′-UTR (CAE 2180 to 2190). This GLUT-1 CAE 2180 to 2190 binds to a trans-acting factor. In the present issue of Circulation Research, Totary-Jain et al report that calreticulin destabilized GLUT-1 mRNA expression in primary bovine aortic endothelial cells and smooth muscle cells under high glucose conditions. They identified calreticulin as a specific destabilizing trans-acting factor that binds to a 10-nucleotide cis-acting element (CAE 2181 to 2190) in the 3′-untranslated region of GLUT-1 mRNA. Their data suggest that CAE 2181 to 2190 – calreticulin complex, which is formed in vascular conditions, is essential for destabilization.

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smooth muscle cells and endothelial cells exposed to hyperglycemic conditions, renders GLUT-1 mRNA susceptible to degradation. This downregulatory mechanism protects vascular cells against damaging effects of an uncontrolled influx of glucose in face of hyperglycemia. The AT1 receptor mRNA decoy studies and the present results indicate the lack of a consensus RNA sequence for calreticulin binding. Instead, a stem-loop structure, rather than a consensus sequence is, proposed as a potential target for calreticulin binding, suggesting the importance of secondary and tertiary structure for protein-mRNA interaction. Other studies identified calreticulin which binds to GC rich stem-loop structure located within the 5’H11032 region of C/EBP H9252 and C/EBP h9251 mRNAs and inhibit translation of C/EBP proteins in vitro and in vivo. The interaction of calreticulin with the stem-loop structure of 3’-end of the rubella virus is reported, which consists of a UAUA loop and a GC rich stem, and it implicated a role in regulation of rubella virus RNA replication. RNA–protein interactions have been shown to influence many processes, including translation, RNA stability, mRNA transport and localization, splicing, and polyadenylation.5

### Pathophysiological Implications of Calreticulin in the Cardiovascular System

It is the interesting finding that high glucose augments calreticulin expression in vascular smooth muscle cells and endothelial cells, although the mechanism and pathophysiological significance of these findings in the vascular cells has not yet been investigated.6 Dai et al documented a profound inhibitory effect of intravenous administration of calreticulin on intimal hyperplasia in rat iliofemoral arteries after balloon injury in vivo.7 Because calreticulin can be found in extracellular locations including the blood, and it has been associated with regulation of immune responses, calreticulin has also been implicated in a number of pathological processes.

### Putative Functions of Calreticulin Domains

<table>
<thead>
<tr>
<th>Structural features and function</th>
<th>N Domain</th>
<th>P Domain</th>
<th>C Domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proceeded by the N-terminal signal sequence targeting the protein to the ER lumen</td>
<td>Proline-rich domain</td>
<td>Rich in acidic amino acids</td>
<td></td>
</tr>
<tr>
<td>Highly conserved amino acid sequence</td>
<td>Amino acid sequence similarity to calnexin, calmegin, and CANLUC</td>
<td>ER retrieval signal</td>
<td></td>
</tr>
<tr>
<td>Potential phosphorylation site</td>
<td>Putative glycosylation site (Leishmania protein)</td>
<td>Antithrombotic activity</td>
<td></td>
</tr>
<tr>
<td>Potential glycosylation site (bovine proteins)</td>
<td>Putative autokinase activity</td>
<td>Prevents restenosis</td>
<td></td>
</tr>
<tr>
<td>Inhibits PDI activity</td>
<td></td>
<td>Ca2+/H11001 sensor of calreticulin protein interactions</td>
<td></td>
</tr>
<tr>
<td>Suppresses tumours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibits angiogenesis</td>
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</table>

**Ion binding**

- BindsZn2+
- Binds to the DNA-binding domain of steroid receptor
- Binds to α-subunit of integrin
- Binds rubella RNA
- Interacts with PDI
- Interacts with ERp57
- Weak interactions with perforin

**Molecules binding**

- Binds to a set of ER proteins
- Strong interactions with PDI
- Strong interactions with perforin
- Lectin-like chaperone site
- Binds Factor IX and Factor X
- Binds to cell surface

**ER**: endoplasmic reticulum

**PDI**: protein disulphide-isomerase

Reprinted with permission from Michalak et al.2 PDI indicates disulphide-isomerase.
The calreticulin gene knock-out study also indicates that the protein plays a role in the development of the heart. It is shown that carticulin is upregulated in the heart during the middle stages of embryogenesis, whereas it is expressed at a low level after birth. Further studies are required to unravel the pathophysiological roles of calreticulin in the pathogenesis of various diseases.

References

KEY WORDS: calreticulin ■ mRNA ■ GLUTs
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