In Memoriam of Professor Witold Drabikowski session:
Calcium and motility

Lectures

LWD.1

EF-hand structure and the role of magnesium in calcium signaling

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The $\text{Ca}^{2+}$-binding helix-loop-helix motif called “EF-hand” is a common structural element of a large family of proteins that function as intracellular $\text{Ca}^{2+}$ receptors. These proteins regulate the activity of their respective target enzymes in response to $\text{Ca}^{2+}$ signals. Importantly, the EF-hand proteins must recognize micromolar concentrations of $\text{Ca}^{2+}$ in the presence of 100–1000 fold excess of the chemically similar divalent cation $\text{Mg}^{2+}$. The intracellular free $\text{Mg}^{2+}$ concentration is tightly controlled in a narrow range of 0.5–1.0 mM, which at the resting $\text{Ca}^{2+}$ levels is sufficient to saturate fully or partially the $\text{Ca}^{2+}$-binding sites of many EF-hand proteins. Since the regulatory function of EF-hand proteins depends on their ability to change the conformation upon binding $\text{Ca}^{2+}$, it follows that the conformational response to $\text{Ca}^{2+}$ must be different from that induced by $\text{Mg}^{2+}$. Several lines of experimental evidence support this view. Our data on troponin C and calmodulin indicate that $\text{Mg}^{2+}$-binding stabilizes the closed-domain conformation in these proteins in contrast to $\text{Ca}^{2+}$, which induces domain opening. This remarkable functional specificity can be explained on the basis of our recently proposed two-step mechanism of $\text{Ca}^{2+}$-binding to the EF-hand, the EF-hand-β-scaffold model (EFBS). The EFBS model emphasizes the importance of the structure connecting the $\text{Ca}^{2+}$-binding loops in a two-EF-hand domain. The invariant backbone carboxyl oxygen ligand in the -Y metal coordinating position, which is a part of the β-scaffold, defines the position of the bound $\text{Ca}^{2+}$. Bond rotation in the β-scaffold enables the movement of the rigid C-terminal part of the $\text{Ca}^{2+}$-binding loop that drives the conformational change. The $\text{Ca}^{2+}$/Mg$^{2+}$ functional specificity of the EF-hand proteins results apparently from the stereochemical constraints imposed on the metal coordinating ligands by the β-scaffold. Similar constraints are expected to occur in most if not all EF-hand proteins, since the EFBS model appears to be applicable to all EF-hand structures irrespective of the type of the $\text{Ca}^{2+}$-induced conformational change (Grabarek Z, 2006, J Mol Biol 359: 509–525). Thus, it is proposed that $\text{Mg}^{2+}$ may play an active role in the $\text{Ca}^{2+}$-dependent regulation of cellular processes by stabilizing the off state of some EF-hand proteins, thereby inhibiting their downstream activity. Deficiencies in the intracellular $\text{Mg}^{2+}$ may cause excessive response to $\text{Ca}^{2+}$ signals, which may lead to pathological conditions.

LWD.2

Calcium buffering chaperones

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Various cellular functions are regulated by changes in cytoplasmic $\text{Ca}$, including gene transcription and expression, protein synthesis, modification and folding, secretion, cell motility, cytoplasmic and mitochondrial energy metabolism, cell cycle progression and apoptosis. Endoplasmic reticulum (ER) is an important Ca storage organelle involved in virtually every aspect of Ca homeostasis. The ER is also a processing place for the maturation, folding, transport and storage of proteins. Calreticulin is an ER resident Ca binding chaperone involved in regulation of intracellular Ca homeostasis and ER Ca capacity. Calnexin is a type I integral membrane chaperone of the ER. Calreticulin and calnexin, together with ERp57 constitute the calreticulin/calnexin cycle that is responsible for the folding and quality control of newly-synthesized glycoproteins. Calreticulin and calnexin have similar substrate specificity and share several common features. Yet, surprisingly, mice bearing a disruption in the calreticulin gene die from a lesion in cardiac development and develop significant metabolic problems whereas calnexin-deficient mice are born alive with myelination problems. In mice, calreticulin deficiency is lethal in utero because of the compromised Ca storage capacity in the ER and disrupted InsP$_3$ receptor-mediated Ca release. This results in impaired cardiac development due to inhibited Ca-dependent transcriptional pathways. Calreticulin and the ER are key upstream elements of calcineurin in Ca-signaling pathways. In contrast, up-regulation of calreticulin leads to impaired development of cardiac conductive system or development of dilated cardiomyopathy. ERp57, an ER associated oxidoreductase, may also be involved in modulation of Ca homeostasis from the lumen of the ER. These observations indicate that molecular chaperones calreticulin and calnexin, and the folding enzyme ERp57, in addition of being involved in protein folding, perform other distinct functions including regulation of Ca homeostasis and signaling during embryonic development.