(IJIRSE) International Journal of Innovative Research in Science & Engineering
ISSN (Online) 2347-3207

Structural and Functional Aspects of Calpain, NCX and PKC in the Calcium Dependent Signaling Platform

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Abstract—**Calpain, the Ca^{2+} dependent cysteine protease can influence overall cellular protein degradation and modify activities of various signaling molecules. The dependency of it on the Ca^{2+} makes them respond to Ca^{2+} signals, resulting in selective downstream cellular targets such as NCX and PKC. Under normal condition NCX1 predominantly localized in the plasma membrane, plays an important role to efflux Ca^{2+} from the cytosol via forward-mode NCX and its inhibition produces dysregulation of Ca^{2+} dynamics with sustained increase in $[Ca^{2+}]_i$. The increase in intracellular calcium is also involved in PKC signaling pathways, where as PKC is the specific target for calpain. Therefore it may conclude that calpain, NCX and PKC crucially involved in the calcium signaling pathways. In this review the structural and functional role of Calpain, NCX and PKC has been focused in terms of calcium dependent cellular signaling.**

Key words-Calcium; Calpain; Exchanger; Kinase; Signaling.

I. INTRODUCTION

An appropriate cytosolic Ca^{2+} concentration is very essential for the tuning of cell biological activities, which are actively associated with Ca^{2+} transporters in the cells [1]. The high affinity pumps such as, SERCA and PMCA are involve to keep $[Ca^{2+}]_i$ levels low, whereas NCXs are normally functional as to expel Ca^{2+} or to bring it into the cell depending on the ion gradient. Each member NCXs are responsible to a distinct activity [1]. Although the functions of NCX in the regulation of intracellular Ca^{2+} homeostasis has been studied so far. But the current research demonstrated that NCX is also functional under the physiological resting condition and play a key role in the removal of cytosolic Ca^{2+} [1]. The cytosolic calcium concentration is a crucial event for the calcium signaling pathways, where calcium dependent proteases such as calpain are tune on. Apart from intracellular calcium level, the calpain activity is also tightly regulated by its endogenous inhibitor, calpastatin association, binding to phospholipids, autoproteolysis, phosphorylation and other protein such as PKC (Protein kinase C) association [2, 3]. PKC isoforms constitute an important component of the signal transduction pathway in respond to a variety of stimuli. Translocation to a distinct intracellular site is an essential step for activation of PKC isoforms [4, 5]. It has been shown that translocation to the membrane site elicit a variety of functions, for example, NF-ƙβ, matrix metallo protease activation. Such translocation of PKC may result of its active fragment upon calpain mediate cleavage [5, 6]. Furthermore, PKC activators have been reported to dramatically alter the morphology and functional activity of cell organelles, where the processing of multiple receptor-dependent signaling events occurs [7].

II. INTERCELLULAR CALCIUM AND CALCIUM HOMEOSTASIS

Besides stabilizing rigid tissues, maintaining cellular integrity and cell adhesions, Ca^{2+} also play fundamental role in cell physiology [8-11]. The normal $[Ca^{2+}]_i$ is about 100-150 nM [12], which is definitely lower than that exist outside of the cell. A numerous facts that increase in $[Ca^{2+}]_i$ could lead to organelle damage, degradation of nucleic acids/proteins and precipitation of phosphates, mitochondrial abnormalities. To keep away from these cellular terrorization efficient Ca^{2+} regulatory mechanisms such as Ca^{2+} influx/efflux and buffering systems turn on to maintain physiological $[Ca^{2+}]_i$ levels [13]. The question may arise in this situation that why Ca^{2+} not other intracellular ions? The choice of Ca^{2+} as a universal intracellular messenger in respect to other cations is credited to its favorable chemical properties. The ionic radius of Ca^{2+} is much larger than that of Mg^{2+} and Zn^{2+} like divalent cations, therefore, Ca^{2+} coordination is more flexible to protein C-end binding [14,15]. Kinetic studies also results that the rate of association/dissociation of Ca^{2+} in comparison to Mg^{2+} is faster [16]. It can associates with

low molecular weight metabolites, membrane phospholipids and other Ca^{2+} transporters, making it a preferred regulator switch [8].

III. CALPAINS

During the mid of 1960s calcium-dependent proteolytic activities were detected by the scientists in the brain, lens and other tissues, and after a long journey such compounds were isolated and characterized. The early name 'calcium-dependent neutral protease' has now been replaced by 'calpain'. The name calpain comes from calcium ‘cal’, for calcium binding property, and papain ‘pain’, a plant thiol protease [17]. But surprisingly the peptidase units of the calpain show least similarity in sequence to that of papain (Fig. 1). Fasta format of sequences of m-calpain, papain and caspase-2 (UniprotKB ID P17655, P00784, and P42575, respectively) represent pair-wise alignment as in figure 1 by SIB permitted toll SIM and graphical viewer tool “lalnview”. Milli-calpain was first identified in rat brain followed by purification and partial characterization [18, 19]. The ubiquitous μ- and m-calpains are known to form heterodimers with the small subunit (Fig. 2), and are named according to their calcium requirement *in vitro* actiation, although both isoform often showed close properties (Table 1).

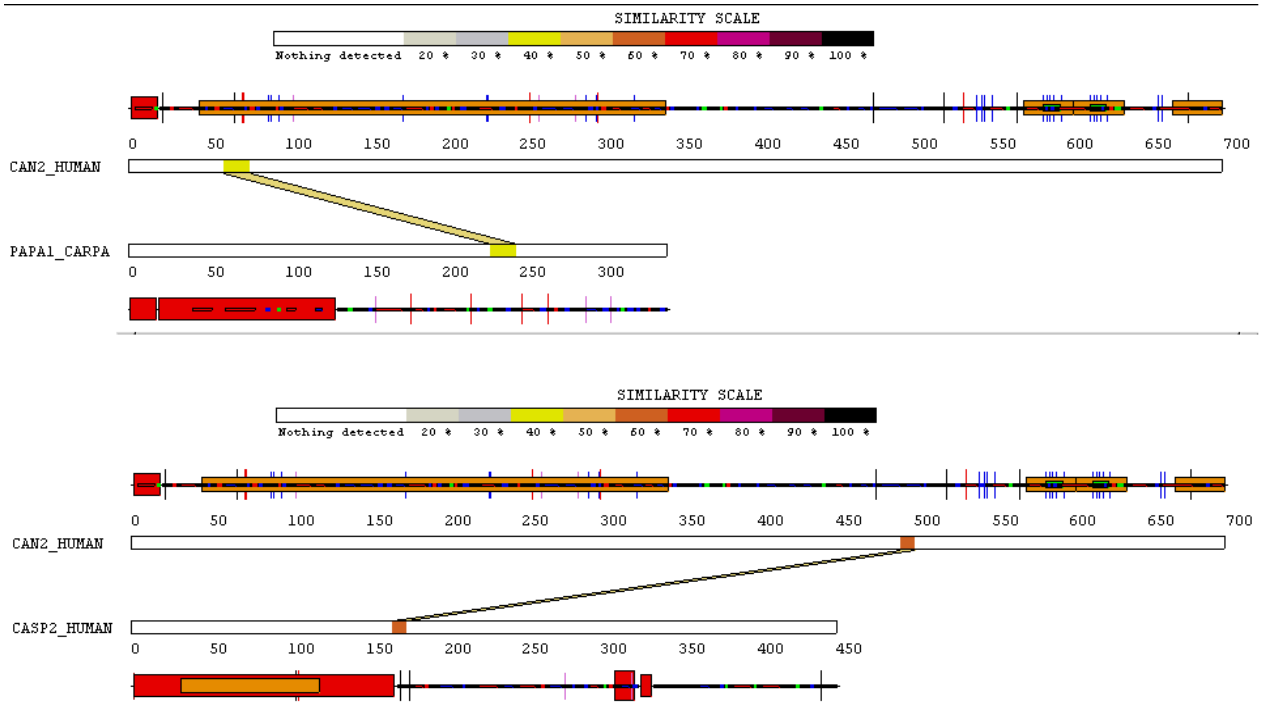

Fig 1: Sequence alignment study. Calpain-2, papain and caspase-2 using SIM tool and lalnview tool, provided by SIB data portal.

Property	**Autolyzed μ-calpain**	**μ-Calpain**	**Autolyzed m-Calpain**	**m-Calpain**
Molecular Mass	99,292	110,188	98,530	108,304
Molecular Mass, large subunit	78,914	81,890	78,152	80,006
Molecular Mass, small subunit	20,396	28,316	20,396	28,316
pH optimum	7.6	7.5	7.4	7.6
[Ca^{2+}] for half-maximal activity	190-210 μM -Phosphatidylinositol 140-150 μM +Phosphatidylinositol	40-50 μM (For proteolysis of casein)	740-780 μM -Phosphatidylinositol 370-400 μM +Phosphatidylinositol	700-740 μM (For proteolysis of casein)
[Ca^{2+}] for binding Calpastatin	0.042 μM	42 μM	24 μM	400 μM
Theoretical pI	5.31	5.36	4.90	4.94

Table1**:** Tabulated form of comparative properties of most common calpain isoforms. (Data resource http://ca.expasy.org/tools/pi_tool.html)

A. The mechanism of activation of calpain by calcium

The Cys and His residues of the calcium dependent proteases are most important for the regulation of their activities. These residues in the active site of papain remains at a distance of 3.7 Å, a distance that allows the His to deprotonate the Cys-SH and increases its nucleophilicity. But in the crystallographic structure of calcium free m-calpain that distance is ~10.5 Å. This distance is too much for acid-base catalysis, which may be the cause of inactivation of calcium free calpain [20]. The protease core of μ-calpain was shown to bind two calcium ions, one in each of DI and DII (Fig. 2). They bind cooperatively to peptide loops, which in turn cause conformational change to the protease core that made Cys and His residues within 3.7 Å. Calpain was early thought to bind one or more calcium ions to the acidic loops, but this is not found in the recent structure of calcium bound inactive m-calpain. Up to four calcium ions bind to PEF domain VI in the small subunit and by inference the same number were thought to bind to the homologous PEF domain DIV in the large subunit [21] (Fig. 2).

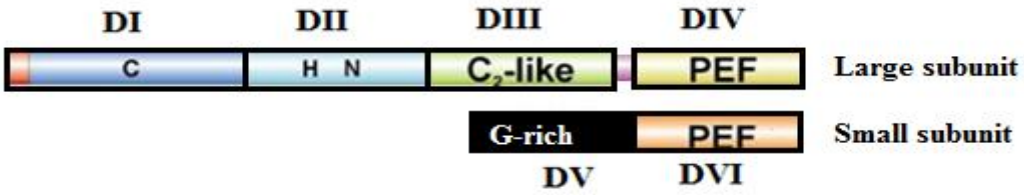

Fig. 2: Domain arrangement of classical calpains.

The structural changes upon calcium binding do not occur within each domain, but in the relative positions of the domains to each other, results a more compact protein. In addition, the two PEF domains (IV and VI) are shifted towards the active site, which displaces the N-terminal anchor helix [21].

B. Calpain regulation by others

Except under pathological conditions, the level of calcium required to activate calpains *in vitro* do not exist within living cells, which led towards the idea that other regulatory mechanisms must involved to lower this calcium

requirement. The binding of phospholipids decreases the calcium requirement for calpains *in vitro* [22, 23], but the *in vivo* relevance of this is a big question. Similarly, regulation of protein-protein interactions changes the calcium requirements of calpains [24], but yet their roles in activation are not clearly understood. Overexpression of calpastatin in cells can decrease calpain activity. Structural and biochemical data indicated that calpastatin preferentially bind to calcium-activated calpains [25, 26], suggesting that this is an attenuation mechanism rather than prevention. It has been proposed that the calpain system developed for high requirement of calcium to provide as a safety device to prevent potentially destructive hyperactivity of calpains, and that it is preferable for calpains to work at less than half-maximal activity. In addition, phosphorylations at several sites control the activities of calpains. Calpain 2 is activated by phosphorylation of Ser^{50} by the ERK mitogen-activated protein (MAP) kinase during migration of fibroblasts and in keratinocytes stimulated with epidermal growth factor [27-29]. Phosphorylation of m-calpain at this site is particularly interesting since μ-calpain, which does not contain a phosphorylation site in this region [28, 29]. Instead, μ-calpain is important for IP-9-induced motility, which requires intracellular calcium flux [29]. By contrast, EGF-mediated activation of m-calpain by phosphorylation occurs in the absence of increased calcium levels. Together, these data suggest that calcium and growth factor-mediated phosphorylation can independently activate calpains in an isoform-specific fashion. Interestingly, only membrane proximal m-calpain is activated by ERK-mediated phosphorylation [30], which suggests that there are alternative pathways of activation for certain m-calpain.

C. Autolysis and aggregation

Structural changes of calpains occur due to binding of calcium in the protease core as well as domains IV and VI and thereby activating the enzyme. Upon calcium binding, autolysis and aggregation occur within the calpains. The event of autolytic cleavage is well documented and effectively lowering the calcium requirement of m-calpain from (400-800) μM to (50-150) μM [31]. The calcium requirement of μ-calpain is not lowered until the autoproteolytic release of DV of the small subunit when it is decreased from 3-50 μM to 0.5-2.0 μM [32]. Further autolysis occurs in flexible loops between DII and DIII, which releases a calcium-dependent weakly active protease core. Structural studies have shown that calcium binding causes a conformational change in which hydrophobic patches are unveiled in domains IV and VI, providing possible sites for aggregation [33].

D. Calpain substrates and proteolysis

The calpain substrates may be transcription factors, membrane receptors, signaling enzymes and cytoskeletal proteins. Calpains mediates extensive degradation of some of these substrates; most are cleaved by a limited manner via highly specific recognition site, results stable fragments. However, no single consensus sequence has been found to have significant value for predicting a protein to be proteolyzed by calpains or even where it cleaves. One hint as to how calpains might affect cell motility comes from the fact that numerous adhesion complex components and migration-related proteins are substrates for calpains [34, 35]. Although proteolysis events of most of these molecules have been demonstrated but several issues still remain unclear and difficult to determine. Further complicating issue is the fact that most of the substrates can be proteolyzed by either μ-calpain or m-calpain, which expressed differing in subcellular localizations and cell-types. Some bioinformatical tools like CaMPDB [36], GPS-CCD 1.0 [37] and LabCaS [38] developed recently to predict the calpain mediate cleavage sites within its substrates.

IV. SODIUM-CALCIUM EXCHANGERS (NCX)

NCX is present in the plasma membrane of most cells and working as bidirectional mode depending on cell physiology. NCXs are members of the large family of Ca^{2+} anti-porter and exchanges 3 Na^{+} by the mean of each Ca^{2+} [39, 40]. Although sodium-calcium transport was first described in the heart and in the squid axon [41, 42], but the actual NCX protein was cloned from canine heart [43]. The exchanger's function was initially shown to be in trans sarcolemmal Ca^{2+} efflux, allow it for rapid Ca^{2+} transport during excitation-contraction mechanism and restoration of cellular Ca^{2+} homeostasis [44], where Na^{+}/K^{+}-ATPase dependent Na^{+} gradient play an important role [45].

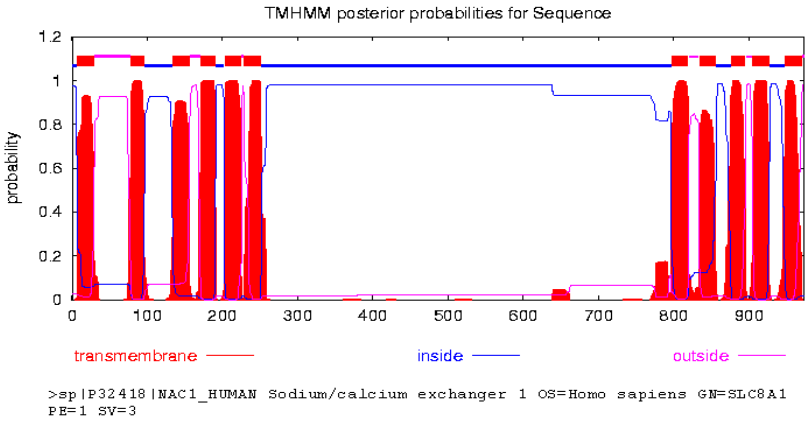

Fig 3: Prediction of NCX1 arrangement in membrane. TMHMM bioinformatics server used to analysis after fasta sequence retrieval form UniProtKB data sources.

A. NCX expression

NCX1 has been most extensively studied in cardiac myocytes and appears to be localized in the T-tubules [43,46]. It has been shown that NCX1 is associated with spectrin-actin cytoskeletal protein via ankyrin [47]. It has also been suggested that NCX1 is in close proximity to the L-type channels, ensuring the control of Ca^{2+} transport and signalling between the proteins involved in Ca^{2+}-induced Ca^{2+} release [48]. A NCX system has also been identified in mitochondrial membrane, where it plays a role for intra-mitochondrial Ca^{2+} homeostasis [49, 50]. NCX1.1, a NCX variant predominantly expressed in cardiac tissues involved in the regulation of cell motility, contraction and proliferation [51]. Arterial smooth muscle cells and endothelial cells are also the NCX expression location [52, 53]. Differential expression of NCX mRNA and proteins has been reported in cerebral cortex, hippocampus and cerebellum [54]. NCX1 appears to be expressed in neuronal cell bodies and dendrites, whereas NCX2 expressed in glial cells, and NCX3, in a restricted subpopulation of neurons [55, 56]. NCX expression has also been observed in skeletal muscle [57].

B. NCX topology and transmembrane domains

Initial molecular studies of NCX1 suggested an open reading frame of 2910 bases encoding for a 970 amino acid protein with a molecular weight of 108 kDa [43, 45] but current model proposed a 938 amino acid protein consisting of nine transmembrane segments (TMS) and a large intracellular loop connecting TMS 5 and TMS 6 [52]. Bioinformatical study for membrane localization was described with TMHMM server (Fig. 3). Several regulatory domains have been identified in the cytoplasmic loop [58]. The C-terminal end is located intracellularly, whereas the N-terminal is located in the extracellular space with N-linked glycosylation but do not involved in NCX functions [59]. The transmembrane domains of NCX are highly conserved amongst Ca^{2+} transporters and appear to be sufficient to mediate the ion transport process [58]. Among the nine TMS of NCX, two internal repeat sequences forms α1 and α2 repeats. The α-repeat sequences are conserved amongst all NCXs [60, 61]. The α-1 repeat joining it to TMS 3 and the first half of TMS 3, exposed to the extracellular surface of the membrane, whereas the α-2 repeat, located between TMS 7-8, is accessible from the cytoplasm [62]. Some studies have demonstrated that highly conserved aspartic residues within the α-repeats may influence Ca^{2+} affinity [62]. TMS2 and TMS3 are involved to create an ideal environment for ion translocation [63].

C. NCX transport and ionic regulation

NCX has the ability to function in bidirectional transport modes either forward or reverse mode [64]. NCX is present in the plasma membrane of most cells and under physiological conditions, extracellular Na^{+} binds to the transport site, internalized and released into the cell. Then Ca^{2+} binds to the same translocation site, internalized into the membrane and then released to the extracellular space [65]. However, when intracellular Na^{+} levels increase under certain cellular stress such as hypoxia, ischemia/reperfusion injury and heart failure, NCX frequently changes to its reverse mode, causing Ca^{2+} influx [66]. This inward flow of Ca^{2+} can contribute to calcium overload, which may cause cellular structural changes, disruption of membrane integrity and mitochondrial dysfunction [6, 50]. Dysregulation of cellular Ca^{2+} homeostasis is also a common feature in a variety of disorders including stroke,

epilepsy, hypertension and trauma, where calpain has been assumed to be involved [67, 68]. Furthermore, NCX is well known target for the calpain, which may result nearly 75-80 kDa large fragments [69].

The Na^+-dependent inactivation is a partial inactivation of outward exchange current and occurs when Na^+ is applied to the intracellular surface of the cell membrane [70]. The Na^+-dependent inactivation could provide a natural defense mechanism to minimize the Ca^{2+} entry via the reverse mode when intracellular Na^+ level are high. Ca^{2+}-dependent regulation also plays an important role in the regulation of NCX, as it is not only required for activation of exchange activity but also modulate Na^+-dependent inactivation [65,70]. Calcium-dependent regulation is inactivated in absence of cytoplasmic Ca^{2+}, but can be reversed by the addition of submicromolar concentrations of free $[Ca^{2+}]_i$. The binding of Ca^{2+} causes a conformational change, which in turn influence the ion exchange activity [65]. Ca^{2+}-dependent regulation may play a role in reducing unnecessary ion transport by limiting Ca^{2+} binding and efflux via the Ca^{2+} transport site, but also reducing Ca^{2+} influx via the reverse mode of action by promoting Na^+-dependent inactivation [65,70]. L-type channels mediated Ca^{2+} influx compensated by an equivalent Ca^{2+} extrusion by NCX [71, 72]. The increase in NCX expression not only caused an increase in forward mode activity but also increases reverse mode, thus contribute to $[Ca^{2+}]_i$ overload during depolarization, overfilling the sarcoplasmic reticulum and increasing oscillatory Ca^{2+} release [71,73].

V. PROTEIN KINASE C (PKC) FAMILY MEMBERS AND DOMAIN STRUCTURE

PKC family of serine/threonine protein kinases plays a crucial role in a variety of cellular events including proliferation, differentiation, apoptosis and cell survival. The mammalian protein kinase C family comprises 10 isozymes grouped into three subfamilies based on their primary structure and cofactor dependences: conventional or classical PKC (α, alternatively spliced βI and βII and γ), novel PKC (δ, ε, η, θ) and atypical PKC (ζ, ι/λ) (Fig. 4). PKCμ/D and ν (PKD) and PKC-related kinases (PRK) are also considered as the PKC family of enzymes [74]. The activity of classical isoforms (cPKCs) is stimulated by cofactors diacylglycerol (DAG), Ca^{2+} and phosphatidylserine (PtdSer). Novel PKC isoforms (nPKCs) are Ca^{2+}-independent, but stimulated by DAG and PtdSer, whereas atypical PKCs (aPKCs) are Ca^{2+} and DAG insensitive, but stimulated by PtdSer. Biochemical assays for multiple activation states of protein kinase C was described by various research articles [75]. The single polypeptide chain of all PKC isozymes consists of carboxy-terminal kinase domain and amino-terminal regulatory moiety, connected via a hinge region. Both domains contain conserved (C) regions of extended sequence homology and variable (V) regions [6, 76]. As a multidomain protein, PKC is under acute lipid and protein-mediated conformational regulation of inter domain interactions [76].

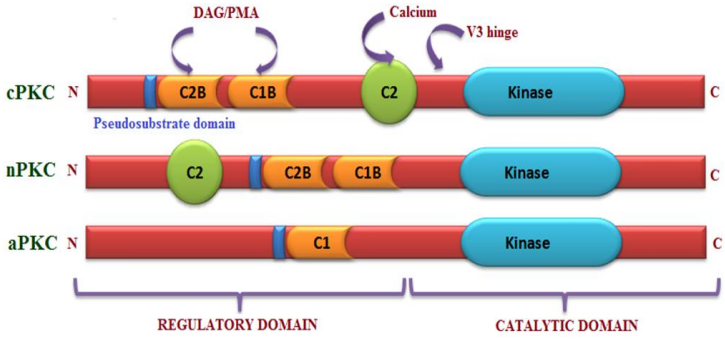

Fig 4: Approximate domain arrangements of Protein Kinase C subtypes.

The regulatory domain consists of pseudosubstrate and two membrane targeting C1 and C2 domains. Pseudosubstrate occupies the substrate-binding cavity of the catalytic domain thereby keeping the enzyme in an auto-inhibited conformation [77]. The C1 domain present in all PKC isoforms is a Cys-rich region that binds the second messenger DAG and phorbol esters and ligand-bound form confers specific binding to PtdSer in the membrane. C1 domain of classical and novel PKCs represents a tandem repeat. Atypical PKCs possess only one copy of C1 domain that does not bind DAG and phorbol esters due to compromised face of the ligand-binding pocket. C2 domain is found in classical and novel PKCs. The C2 domain of cPKCs binds to the anionic lipids in the membrane in a Ca^{2+}-dependent manner, whereas nPKC C2 domain does not bind Ca^{2+} due to the absence of key

aspartic acid residues involved in coordination of Ca^{2+} [74,78]. The highly conserved among PKA, PKB and PKC catalytic domain comprises ATP binding and substrate binding domains. PKC phosphorylates substrates on Ser or Thr residues [74]. Despite the large number of PKC isotypes, substrate promiscuity and the expression of multiple isoforms in the same cell, individual PKC isoforms seem to have unique function. PKC function is regulated by: (a) phosphorylation (b) catalytic activation by cofactor binding and (c) interaction with PKC binding proteins [79]. Crystal structure analysis of PKCα-C2 domain associated with Ca^{2+} and PtdIns(4,5)P_2 was represented in Figure 5 (rcsb pdb code 3GPE).

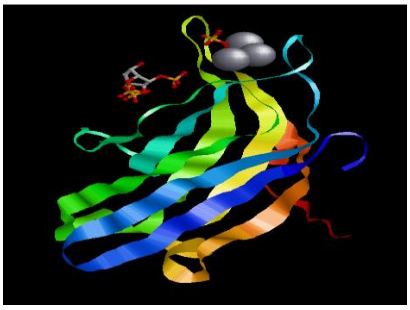

Fig. 5: Calcium and PtdIns (4,5)P_2 bound structure of PKCα. Data resource PDB, viewer tool RasMol 2.7.5 (RCSB PDB ID 3GPE).

A. PKC activation

PKC could be activated by a number of stimuli such as hormones, neurotransmitters or growth factors by the mean of hydrolysis of membrane lipids [80]. Conventional PKCs are activated downstream of receptor tyrosine kinases (RTK), nonreceptor tyrosine kinases as well as G-protein coupled receptors (GPCR) that activate phospholipase C (PLC)-mediated hydrolysis of PtdIns(4,5)P_2 to DAG and IP_3 [81, 82]. The IP_3 induces Ca^{2+} release from endoplasmic reticulum (ER) [83]. Elevated intracellular Ca^{2+} recruits PKC to the membrane via C2 domain. Conformational change induced by membrane targeting able to release the pseudosubstrate from the active site and in that way active conformation allowing substrate binding and catalysis to proceed. The active PKC is highly sensitive to phosphatases and that the dephosphorylated PKC is targeted to proteolysis by ubiquitin or caveolin-dependent mechanisms [6, 84, 85]. Binding to the molecular chaperone Hsp70 allows backing to the pool of functional PKC [77]. The regulation is much more complicated through the catalytic mode of activation, which occurs by a variety of lipids and second messengers such as DAG, fatty acids, ceramide, cholesterol sulfate, phosphatidic acid, PIP_3 etc. Lipid-protein interactions mediated translocation and subcellular distribution has been shown to occur by the process of PKC activation [86].

B. PKC expression and translocation

Different cell types express their own complement of PKC isoforms [87], but some isoforms such as PKCα are ubiquitously expressed and may be involved in the regulation of a variety of cellular functions. Various PKC isoforms identified in vascular smooth muscle are involved in cell proliferation and differentiation [6, 88, 89]. During cell stimulation the inactive form of PKC is thought to be distributed throughout the cytosol or to be localized to specific regions of the cell [5, 6]. Hence, the regulation of PKC activation and its translocation to isoform-specific sites are the recent areas of research interest. In stimulated cells, PKC isoforms may translocate from inactive pools to a specific region where they can activate. Therefore, the translocation of PKC may represent a major mode of control of isoform functions [5, 6]. All classes of PKC isoforms translocate to the cell membrane thereby allowing access to lipid co-factors required for enzyme activation. Colocalization studies have indicated the association of different PKC isoforms with components of the actin filament [90], microtubules [91] of the cytoskeleton in different cell types. Activation of PKC results phosphorylation of various cytoskeletal proteins, and it has been reported that PKCα isoforms is translocated from the cytosol to the cell membrane and nucleus in response to stimulation by a variety of hormones and growth factors [5, 6, 92, 93]. In summary, the translocation signaling movements of a specific isoform are not fixed but may vary significantly with the cell type, stimulations and other regulatory factors.

C. PKC activation by calpain

Indeed, under resting conditions $[Ca^{2+}]_i$ is insufficient to activate PKC but upon corresponding receptor stimulation and successive Ca^{2+} mobilization, the $[Ca^{2+}]_i$ increases transiently but adequate to promote translocation of inactive PKC to the plasma membrane for activation [5,6,92], this event accelerate the affinity of PKC for Ca^{2+} [94]. The calcium dependent PKCs are normally activated in response to receptor activation. In other hand, calcium can also activate calpain like proteases. By the way this activated protease can cleave PKC in a restricted manner [6, 69]. Thus, calcium ions can induce the PKC signaling and/or by calpain-mediated cleavages of PKC [5, 6]. It is well known that some PKC isoforms can be proteolytically cleaved at the V3 region (Fig. 4) by the calcium dependent proteases, such as calpain to generate a cofactor-independent free catalytic subunit (PKM) [5, 6, 95]. Bioinformatical software such as GPS-CCD, CaMPDB and LabCas mediated cleavage site prediction also shows results in support of this with high frequency [36-38]. Therefore, calpains mediated cleavage of PKC has been considered to an alternative mechanism for activation of PKC. Ischemia prompts a drastic increase in intracellular calcium levels and boosts the activities of PKC and calpain together [96]. Over expression of calpain under ischemic conditions may lead selective cleavage of PKC. The increased abundance of the active C-terminal leads to hyperphosphorylation of normal PKC target substrates, for example, MARCKS may be responsible for several types of cellular dysregulations [5]. PKC interacts transiently with membranes in the absence of calcium or DAG. PKC isoforms also translocate to specialized membrane compartments [5, 6], and caveolin-bound PKC represents a membrane-associated enzymes hanging for activation [97].

VI. CONCLUDING REMARKS AND FUTURE DIRECTION OF WORK

The present review emphasizes on the intracellular calcium and its regulation in terms of Na^+/Ca^{2+} exchanger, calpain system and Protein Kinase C functions, an emerging area of current research. Although we have reported intracellular cellular/ intra caveolar (a plasma membrane invagination) calcium concentration is increases through the NCX [69] and m-calpain-protein kinase Cα axis eliciting a role of low dose of ouabain in the cell proliferation [6], But elucidation of the role of calmodulin dependent protein kinase, known to be activated by an increase in $[Ca^{2+}]_i$, in this scenario remains to be need of research. Involvement of $p^{44/42}$MAPK (ERK1/2) isoforms in this regard needs to be examined preciously, because it has a crucial role in the regulation of cell growth. An important question could be raised about the mechanism(s) by which calpain and specific PKC isoform activation occurs within oxidant environment. Furthermore recently we have reported that low dose of ouabain able to increase $[Ca^{2+}]_i$ without significant inhibition of Na^+/K^+ -ATPase, which in turn activated the m-calpain [6]. Therefore it's a general quarry that how this increase in $[Ca^{2+}]_i$ occurs. Hence, functional regulation of calcium signaling in several pathophysiological conditions is an important aspect of future research.

ACKNOWLEDGEMENT

I would like to thank University Grant Commission, Govt. of India, New Delhi for the financial assistance.

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