PREFACE

In *Calmodulin: Structure, Mechanisms and Functions*, the authors consider small and poorly-studied groups of plant calcium-dependent protein kinases that directly interact with calmodulin molecules. In plants, Ca2+ activates calmodulin-like domain kinases that do not require calmodulin or phospholipids. Thus these kinases differ from both CaMK and PKC families prevalent in mammalian cells.

Next, various strategies of purification of recombinant proteins using CaM-based purification systems are reviewed and discussed. Protein purification is a crucial process in biotechnology industries and life science research laboratories. Amongst these purification strategies, affinity purification has garnered a lot of attention due to its higher speed and selectivity, leading to enhanced purity in fewer steps.

In the closing chapter, the authors describe atomic-level structures of Ca2+-bound CaM (Ca2+/CaM) bound to the PSD-95 N-terminal domain. The N-lobe of CaM forms a cap that binds to the N-terminus of PSD-95 and sterically blocks the palmitoylation of PSD-95 at Cys3 and Cys5. The CaM C-lobe forms hydrophobic contacts with PSD-95 residue Y12, and the Y12E mutation abolishes Ca2+-induced postsynaptic release of PSD-95.

Chapter 1 - Ca^{2+} plays a fundamental role as a second messenger in living cells and calmodulin (CaM - Calcium-Modulated protein) is a

universal Ca^{2+}-sensor in eukaryotes. CaM is one of the most conserved proteins in all eukaryotes; nevertheless, there are some differences of Ca^{2+} signaling in plants. The review is dedicated to the consideration of small and poorly-studied group of plant calcium-dependent protein kinases that directly interact with calmodulin molecules. In plants Ca^{2+} activates calmodulin-like domain kinases (CDPKs) that do not require calmodulin or phospholipids. Thus these kinases differ from both the CAMK and PKC families prevalent in mammalian cells. However, there are few small groups of plant protein kinases that need calmodulin: nonreceptor CDPK-related kinases (CAMK1-8) and calmodulin-binding receptor-like cytoplasmic kinases (CRCK1-3); receptor-like - calmodulin-binding receptor kinase CAMRLK (CARLK) and CaM-binding LRR receptors - somatic embryogenesis receptor kinases (SERK1 and SERK2), phytosulfokine receptors (PSKR1 and PSKR2), brassinosteroid LRR receptor kinase 1 (BRI1) and receptor protein kinase CLAVATA1 (CLV1). In addition, actual taxonomic distribution of EF-hand domain containing proteins is presented.

Chapter 2 - Protein purification is a vital step in the workflow of both biotechnology industries and life sciences research laboratories. Amongst various purification strategies, affinity purification has gained a lot of importance due to its higher speed and selectivity, achieving enhanced purity in fewer steps. Calmodulin (CaM) and calmodulin binding peptide (CBP) form an excellent affinity pair, which has been employed for purification of various recombinant proteins. CaM is a ubiquitous Ca^{2+}-binding protein in eukaryotes, which acts as an intracellular Ca^{2+} sensor. CaM interacts with various downstream target proteins in a Ca^{2+}-dependent manner. Upon changes in Ca^{2+} concentration, CaM changes its conformation and hydrophobicity and consequently changes its interaction with the target CBP. The CaM-CBP affinity pair exhibits invaluable properties, such as a broad range of binding affinities (from pM to μM range), reversible binding in the presence or absence of Ca^{2+}, and its relatively small size and high expression level. Either CaM or CBP can be used as a tag for recombinant expression of proteins of interest in multiple expression systems. Purification can be performed using Ca^{2+}-dependent

affinity and hydrophobicity change of CaM. Following the purification steps, this tag can be either retained or removed based on the specific application of the purified protein. In this chapter, the authors review and discuss various strategies of purification of recombinant proteins using CaM-based purification systems.

Chapter 3 - Calmodulin (CaM) is a ubiquitous EF-hand Ca^{2+} sensor protein that binds and regulates over 50 different target proteins. In the brain, CaM binds to the postsynaptic density 95 protein (PSD-95) and promotes the Ca^{2+}-induced release of PSD-95 from the postsynaptic membrane in glutamatergic synapses. This Ca^{2+}-dependent postsynaptic targeting of PSD-95 is known to control the trafficking and localization of neuronal AMPA-type glutamate receptors (AMPARs). The CaM-induced postsynaptic displacement of PSD-95 is also necessary for the loss of AMPARs during homeostatic scaling down of synapses. In this review, the author describes atomic-level structures of Ca^{2+}-bound CaM (Ca^{2+}/CaM) bound to the PSD-95 N-terminal domain. The N-lobe of CaM forms a cap that binds to the N-terminus of PSD-95 and sterically blocks the palmitoylation of PSD-95 at Cys3 and Cys5. The CaM C-lobe forms hydrophobic contacts with PSD-95 residue Y12, and the Y12E mutation abolishes Ca^{2+}-induced postsynaptic release of PSD-95. In essence, Ca^{2+}/CaM binds specifically to the depalmitoylated and cytosolic form of PSD-95, thereby triggering the release of PSD-95 from the postsynaptic membrane. Phosphorylation of PSD-95 at residue T19 further enhances Ca^{2+}/CaM binding to PSD-95 and causes long term depression (LTD). The recent NMR structure of Ca^{2+}/CaM bound to PSD-95 phosphorylated at T19 revealed that the negatively charged phosphate group at T19 forms an electrostatic contact with the positively charged side chain of K115 from CaM, and PSD-95 residue, E17 forms an intermolecular salt bridge with R126 in CaM. The structure of CaM bound to PSD-95 will be discussed and interpreted in terms of a new model (CaM-palmitoyl switch) that explains how palmitoylation, Ca^{2+}/CaM and phosphorylation each control the release of PSD-95 from the postsynaptic membrane.