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Review

CLIC proteins, ezrin, radixin, moesin and the coupling of membranes to the actin cytoskeleton: A smoking gun?☆

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ABSTRACT

The CLIC proteins are a highly conserved family of metazoan proteins with the unusual ability to adopt both soluble and integral membrane forms. The physiological functions of CLIC proteins may include enzymatic activity in the soluble form and anion channel activity in the integral membrane form. CLIC proteins are associated with the ERM proteins: ezrin, radixin and moesin. ERM proteins act as cross-linkers between membranes and the cortical actin cytoskeleton. Both CLIC and ERM proteins are controlled by Rho family small GTPases. CLIC proteins, ERM and Rho GTPases act in a concerted manner to control active membrane processes including the maintenance of microvillar structures, phagocytosis and vesicle trafficking. All of these processes involve the interaction of membranes with the underlying cortical actin cytoskeleton. The relationships between Rho GTPases, CLIC proteins, ERM proteins and the membrane:actin cytoskeleton interface are reviewed. Speculative models are proposed involving the formation of localised multi-protein complexes on the membrane surface that assemble via multiple weak interactions. This article is part of a Special Issue entitled: Reciprocal influences between cell cytoskeleton and membrane channels, receptors and transporters. Guest Editor: Jean Claude Hervé.

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1. Introduction

Since their discovery, the Chloride Intracellular Channel proteins (CLICs[2]) have challenged our preconceptions of what constitutes an ion channel membrane protein *versus* a cytosolic globular protein [1–10]. Although first identified and cloned as chloride ion channels [9–11], the sequences of CLIC proteins do not resemble those of more conventional ion channel proteins. This has given rise to continued debate as to whether the CLICs function as ion channels or have other, possibly additional, non-channel cellular functions [12–15]. Three things are clear: first, while being largely soluble proteins, CLICs can localise to cell membranes or lipid bilayers under specific conditions. Second, the localisation of CLIC proteins to cell membranes is often associated with processes that involve membrane remodelling mediated by the cortical actin cytoskeleton and third, the CLIC proteins are associated with small GTPases. These three points are the central concerns of this review.

2. The players — description of the characters

2.1. CLIC proteins

The CLIC proteins are a family that is conserved throughout metazoa. In vertebrates, there are usually six CLIC paralogues, CLIC1–6, that have arisen from a single chordate CLIC [13]. These vertebrate CLIC paralogues appear to have diversified in function with some CLICs (CLIC1 and CLIC4) appearing in a wide range of cells while others have a more limited distribution (CLIC5, CLIC6). CLIC-like proteins (usually one) appear to be present in all invertebrate metazoa. The fruit fly, *Drosophila melanogaster*, has a single CLIC-like protein (DmCLIC) [16], while the nematode, *Caenorhabditis elegans*, has two, Exc-4 and Exl-1 [17,18]. The recently sequenced genomes of choanoflagellates [19] (the closest single celled organisms to metazoa) indicate that they also have a CLIC-like gene.

All CLIC proteins contain a ~240 residue CLIC module that adopts a GST superfamily fold [20,21]. This consists of an N-terminal thioredoxin fold followed by an all α helical C-terminal domain (Fig. 1A & B). Unlike most GSTs, most CLIC proteins contain a conserved cysteine residue at what structurally looks to be an enzymatic active site [13,22]. This site resembles that of glutaredoxin, including the sequence motif: Cys-Pro-Phe-Ser/Cys [20]. The active site cysteine (Cys24 in human CLIC1) is conserved in all CLICs with the notable exception of the two CLIC-like proteins in *C. elegans* and related nematodes, where the cysteine is replaced by aspartate [17,18]. There are two additional cysteine residues that are conserved in all CLICs (Cys178 and Cys223, human CLIC1 numbering). As predicted from the crystal structure, redox (reduction-oxidation processes) appears to be important for CLIC protein function [16,23–35].

The traditional view of proteins is that their sequence determines a unique, well-defined three dimensional structure [36]. However, recent work has discovered a growing class called metamorphic proteins that can adopt more than one well-defined three dimensional structure [37–39]. CLICs are metamorphic proteins, being able to undergo reversible conformational transitions to adopt several, stable, well-defined three-dimensional configurations.

Under reducing conditions, CLIC1 is a soluble monomer with a GST fold. Under oxidising conditions, a minor conformer is trapped and populated, revealing a non-covalent dimer, which is stabilised by an intramolecular disulphide bond [33]. The dimer interface is highly hydrophobic implying that a monomer with this conformation would be highly unstable in aqueous solution. The structure of the oxidised dimer reveals a dramatic rearrangement of the N-terminal thioredoxin fold domain where the four-stranded β sheet has been replaced by an all-helical structure (Fig. 1C) [33]. Biophysical studies have shown that the N-terminal domain of CLIC1 is conformationally plastic with its structural stability reducing at low pH [40–43].

CLIC proteins bind to artificial lipid bilayers. Studies using surface plasmon resonance (SPR) have measured the binding of human CLICs (CLIC1 [16] and CLIC4 [32]) and invertebrate CLIC-like proteins (Exc-4 and DmCLIC [16]) to membranes in a concentration dependent manner. Membrane binding of these CLIC and CLIC-like proteins increases with acidic pH and under oxidising conditions [26]. This correlates with the oxidation-triggered structural transition observed in CLIC1 [33] and the conformational instability of CLIC1 at low pH values [40–43].

CLIC proteins can spontaneously integrate into lipid bilayers. While direct integration of a soluble protein into a membrane is not a common property, there are large classes of proteins that possess this ability including bacterial pore forming toxins [44], annexins and the Bcl-2 family of apoptotic proteins [45].

The first identified CLIC protein, originally called p64, was purified to homogeneity and subsequently cloned on the basis of its capacity to form chloride ion channels [9–11]. Since then, several independent groups have demonstrated the integration of CLIC proteins into bilayers using electrophysiological studies based on bacterially-expressed, soluble CLICs and artificial lipid bilayers [16,20,29–33,46–49]. The probability of observing channel activity increases with decreasing pH [16,32,47] and oxidising conditions [16,32]. In the case of CLIC1, mutation of the active site cysteine residue Cys24 to an alanine altered the channel

[2] Abbreviations: CLIC — Chloride Intracellular Channel protein; ERM — ezrin, radixin, moesin family protein; WT — wild type; NADPH - nicotinamide adenine dinucleotide phosphate; ROS — reactive oxygen species; PIP5K — Phosphatidylinositol-4-phosphate-5-kinase; $PI(4,5)P_2$ — phosphatidylinositol 4,5-bisphosphate; FERM — band 4.1, ezrin, radixin, moesin domain; C-ERMAD — C-terminal ezrin, radixin, moesin actin-binding domain; GAP — GTPase accelerating protein; GEF — guanine exchange factor; GDI — guanosine nucleotide dissociation inhibitor; TM — transmembrane; PTM — putative transmembrane region; FRET — fluorescence resonant energy transfer; CRIg — complement receptor of the immunoglobulin.