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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.

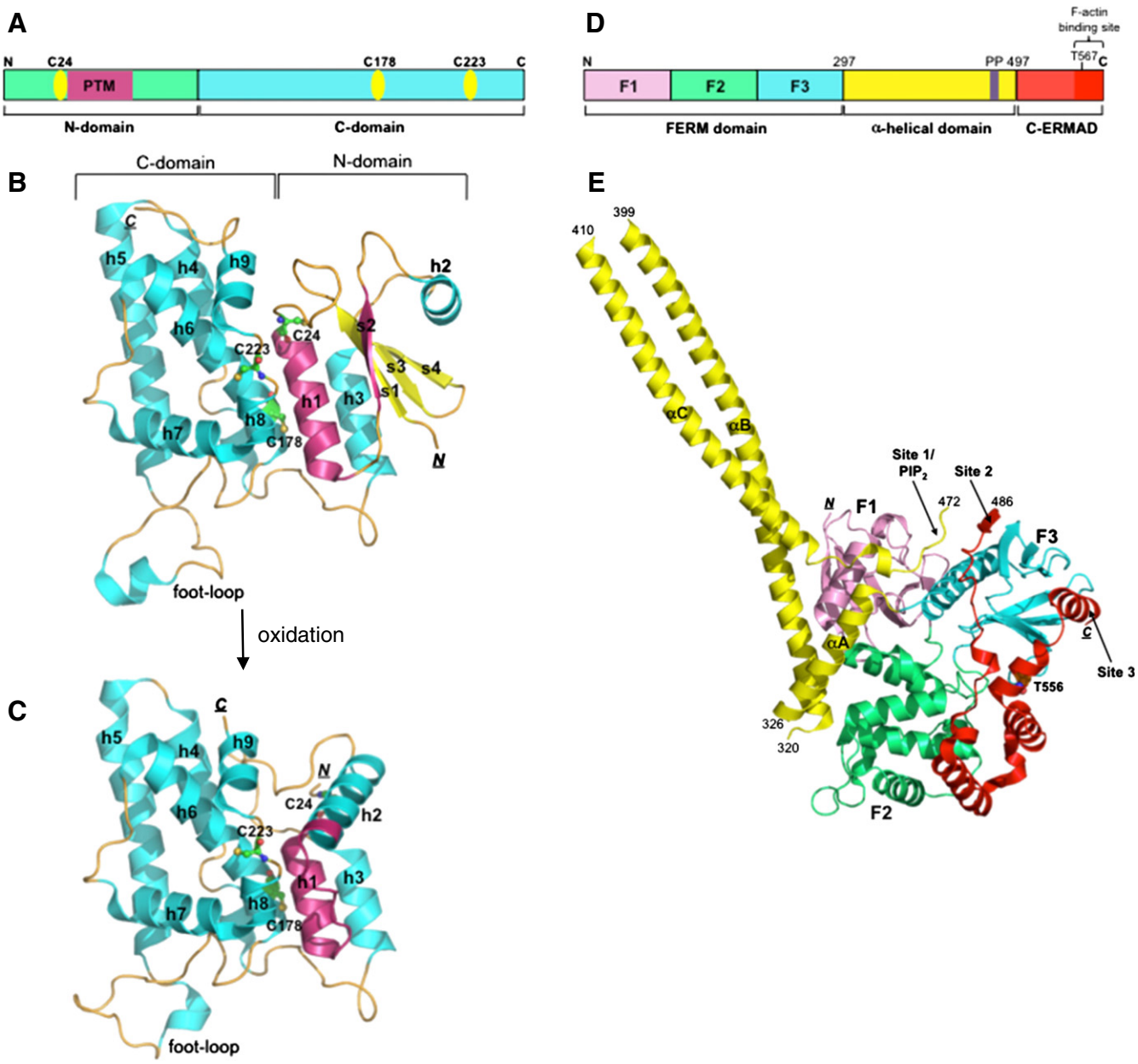

Fig. 1. *CLIC and ERM protein structure.* A: Diagram showing conserved features of CLIC proteins using human CLIC1 sequence numbering. B: Ribbon diagram of the overall crystal structure of reduced, monomeric CLIC1 (PDB ID: 1K0M) showing the GST fold that contains a thioredoxin-like N-terminal domain (β-strands s1-s4 and α helices h1-h3) and an all α-helical C-terminal domain (α helices h4-h9). The PTM region, residues 24–46, (shown in magenta) comprises helix h1 and β-strand s2. The conserved cysteine residues: Cys24, Cys178 and Cys223 (human CLIC1 sequence numbering) are presented as green stick models. C: The structure of the soluble half-dimer produced on oxidising CLIC1. The N-terminal domain of CLIC1 undergoes a radical structural arrangement to an all α-helical domain (PDB ID: 1RK4). D: The domain organisation of ERM proteins using human ezrin sequence numbering. Overall, the ERM proteins consist of: the ~300 residues FERM domain at the N-terminus (where F1, F2 and F3 are the three subdomains of the FERM); the ~200 residue α-helical domain that contains a coiled-coil and a proline-rich (PP) linker region (the proline-rich region exists only in ezrin and radixin); and the ~100 residue C-ERMAD which contains the phosphorylation site (Thr567 in human ezrin) and the F-actin binding site. E: The crystal structure of full-length *Spodoptera frugiperda* moesin (Sfmoesin) in the inactive state (PDB ID: 2I1K), showing the three subdomains of FERM: F1 (pink), F2 (green) and F3 (cyan), the α-helical domain (yellow) and the C-ERMAD domain (red). The three binding sites on the FERM domain are indicated by the black arrows. Site 1 is the $PI(4,5)P_2$ binding site which in the inactive ERM is occupied by the linker region. Site 2 binds the cytoplasmic extensions of integral membrane proteins (such as CD43, CD44, ICAM-1, PSGL-1). Site 3 is the C-ERMAD/NHERF binding site.

conductance and resulted in the CLIC protein being no longer redox sensitive [31].

Despite being poorly selective between anions and cations, CLIC4 also displayed redox sensitivity, as did a truncated N-terminal version of the protein containing the active cysteine [29]. The presence of the reactive site cysteine may be a further factor controlling the transition of the soluble CLIC state to the membrane bound form. Thus, the electrophysiological observation of ion channels follows conditions where the CLIC protein structure is less stable and the proteins have a propensity to bind to membranes.

Insertion of CLIC proteins into membranes is affected by lipid composition [48], especially cholesterol [31,48,50]. Recent studies using tethered bilayer membranes and Langmuir monolayer films show that both the interaction with membranes and the conductance activity due to CLIC1 are sensitive to the presence of cholesterol in the membrane [50]. The addition of cholesterol to soluble CLIC1 prevents this interaction with membranes. This is reminiscent of the spontaneously membrane-inserting bacterial toxins – the cholesterol-dependent-cytolysins – which are believed to interact with membranes via cholesterol-rich rafts [51].

Since the discovery of the CLICs, it has been debated whether CLIC proteins form ion channels by themselves or form elements controlling integral membrane ion channels. For example, CLIC2 has been shown to modulate the ryanodine receptor calcium release channel [52,53], binding to its cytoplasmic face [54]. However, the above studies using recombinant CLIC proteins clearly show that CLICs are indeed channel proteins, at least *in vitro*.

The electrophysiological characteristics of the CLIC channels produced by recombinant proteins and artificial bilayers correspond with the channel properties observed in cells expressing CLIC proteins [20,47]. These include the effects of the chloride ion channel inhibitor indanyloxyacetic acid-94 (IAA94) which was used to both identify and when coupled to sepharose, purify the original CLIC protein (bovine CLIC5B, initially called p64 from the bovine kidney cortex membrane) [9–11]. Thus, although the mechanism is incompletely understood, it is likely that CLIC proteins form ion channels *in vivo*.

Ion channel activity for CLIC proteins has been detected in cells, as well as in artificial systems using purified proteins. When expressed in CHO cells, CLIC1 forms an ion channel in the plasma membrane, spanning the membrane so that the N-terminus is extracellular and the C-terminus cytoplasmic [55]. This was determined by studying the chloride conductance of CHO cells transfected with CLIC1 constructs bearing either an N or C-terminal epitope tag. Ion channel conductance could only be blocked by anti-tag antibodies when on the same side of the membrane as the epitope tag.

Based on sequence analysis, the putative transmembrane domain (PTM) of CLIC proteins is thought to form a single membrane spanning helix in the channel state, corresponding to the region Cys24-Val46 of CLIC1 (Fig. 1A & B) [7–9]. The first experimental evidence for this PTM came with the discovery of CLIC4 which behaved as an integral membrane protein [8]. Proteinase K digestion of intact microsomes containing CLIC4 showed a ~27 kDa reduction in molecular weight on SDS PAGE, leaving ~6 kDa fragment in the membrane, which presumably included the PTM. Complete digestion of membrane bound CLIC4 was only achieved upon treatment with Triton X-100.

Direct evidence for the PTM has come from fluorescence quenching, fluorescence energy transfer (FRET) and electron paramagnetic resonance studies using site-directed cysteine scanning mutagenesis followed by probe labelling. These studies directly show that CLIC1 integrates into membranes [26,35] and that the PTM inserts into the membrane with distance measurements that are consistent with a TM α helix [35]. FRET studies on CLIC1 in membranes have shown that the integral membrane form consists of an oligomer, consistent with a pore formed by the PTM in a TM α helical conformation [35].

Although the evidence above indicates that CLIC1 can adopt an integral membrane form, not all membrane-associated CLIC proteins appear to integrate into membranes. CLIC4 translocates to the plasma membrane of NIE-115 neuroblastoma cells (see Section 4.1, below) [22]. However, the N-terminal epitope tag on CLIC4 did not protrude from the cell [22] as might be expected by analogy to CLIC1 [55]. In a similar vein, the *C. elegans* CLIC-like protein, Exc-4, resides exclusively on the luminal membrane of the excretory cell (see Section 3.2.1, below) [17,18]. However, fusion constructs of Exc-4 that contain N-terminal GFP domains behave like wild type Exc-4 in localising to the luminal membrane and rescuing the cystic phenotype of *exc-4* null animals [17]. This suggests that functional Exc-4 may not need to form a transmembrane protein.

CLICs interact with multiple cytoskeletal components [2,56] and the ion channel activity of several CLIC family members is regulated by cytoskeletal F-actin. When soluble recombinant CLIC1 or CLIC5 were inserted into planar lipid bilayers, their ion channel activity was inhibited by F-actin, whilst that of CLIC4 was not [46].

2.2. ERM: ezrin, radixin and moesin

Like the CLICs, the ERM protein family is conserved throughout metazoan evolution [57] with family members observed in the choanoflagellates [58]. In vertebrates, there are three paralogues, ezrin, radixin and moesin, hence the acronym ERM [59–61]. In non-vertebrates there is usually only a single ERM protein. Although their names differ, the proteins show high sequence identity (73–81% among human ERM). ERM proteins are closely related to the NF2 tumor suppressor protein, Merlin, which shares the same architecture and is also involved in membrane-cytoskeletal regulation [58].

ERM proteins have a three domain structure: a ~300 residue N-terminal FERM domain [62,63]; a ~200 residue central α helical coiled-coil domain which is sometimes followed by a proline-rich linker; and a ~100 residue C-terminal domain (called C-ERMAD) that contains an F-actin binding site and a threonine (Thr567 in human ezrin) which is phosphorylated on activation (Fig. 1D). The structure of an inactive full-length ERM from the insect *Spodoptera frugiperda* has been determined [64] (Fig. 1E) along with mammalian ERM structures of the active FERM domains only of moesin [65], radixin [66] and ezrin [67], and the dormant (inactive) complex of the FERM:C-ERMAD domains of human moesin [68].

ERM proteins exist in two states: the dormant autoinhibited state and the active state [69] (Fig. 2). In the dormant state, ERM is biologically inert, as the functional binding sites on both the FERM and C-ERMAD domains are masked by intramolecular interactions between the domains [70]. On activation, these domains separate exposing functionally important binding sites on both FERM and C-ERMAD domains.

The mechanism of activation of ERM proteins is only partially understood. Two key steps are the phosphorylation of the conserved threonine in C-ERMAD and the binding of the phospholipid phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) by the FERM domain [71–75]. These steps may be sequential, with $PI(4,5)P_2$ binding preceding phosphorylation [75]. Thr567 (human ezrin numbering) has been identified as the conserved C-ERMAD residue whose phosphorylation coincides with activation [76]. On phosphorylation of Thr567, the affinity of C-ERMAD for the FERM domain is reduced while its affinity for F-actin remains unaltered [77,78]. Several kinases have been implicated in ERM activation including: Rho-kinase (ROCK) [77], protein kinase Cθ [78,79] and protein kinase Cα [80].

In the active state, ERM proteins link membranes to the actin cytoskeleton. The free C-ERMAD binds to actin filaments [81]. The structure of this complex is not known, however, it has been suggested that the last two residues of ezrin C-ERMAD are critical for interaction with both FERM-domain in the dormant state and F-actin in the active state [69].

On activation, the FERM domain can bind to membranes via multiple interactions. The FERM domain can bind directly to membranes via binding specifically to the phospholipid $PI(4,5)P_2$ (Fig. 1E, labelled "Site 1"). It also binds directly to the cytoplasmic extensions of membrane proteins [58] including: integrins, G protein coupled receptors (GPCRs), cadherins, sialoglycoproteins (CD43 [82], CD44 [83]), adhesion molecules (ICAM [84], VCAM), ATPases, selectin receptors (PSGL-1 [85]) and neutral endopeptidase [86] (Fig. 1E, Site 2). Finally, the FERM domain binds to scaffolding proteins that, in turn, bind to the cytoplasmic tails of other integral membrane proteins [87–89] (Fig. 1E, Site 3).

Scaffolding proteins that bind ERM include: EBP50, also known as NHERF1, and NHERF2 [70,90,91]. Both of these proteins consist of two PDZ domains followed by a C-terminal ERM binding domain [92]. In the dormant state of the scaffolding protein, the ERM binding domain interacts with its own PDZ domain. On activation, the C-domain binds to ERM while the two PDZ domains bind either the cytoplasmic tails of integral membrane proteins or to other scaffolding proteins, which, in turn, bind membrane proteins (Fig. 2).

ERM proteins play a key role in endocytosis, phagocytosis [93,94], vesicular trafficking and vesicle maturation [95]. Their function is to organise the cortical actin structures so that they engulf particles or cells, relocate the vesicles to sub-cellular regions and facilitate vesicular fusion. In this role, they cooperate with Rho GTPases acting as both upstream and downstream RhoA effectors [96].

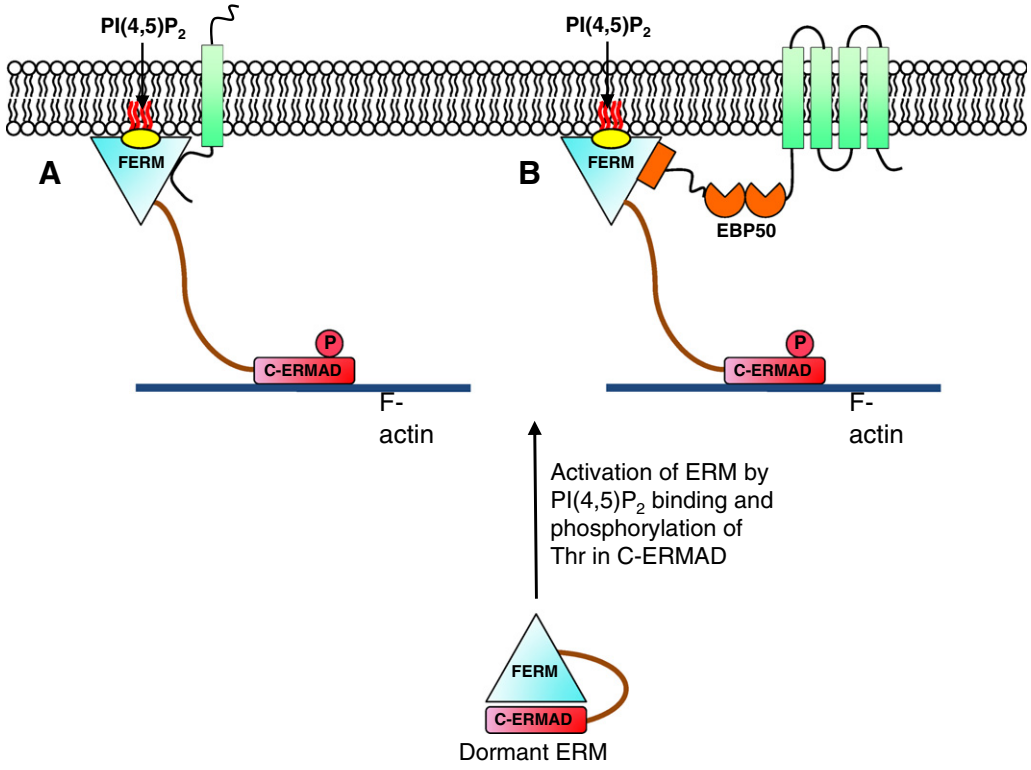

Fig. 2. The activation of ERM proteins. The ERM proteins exist in two states: the dormant/inactive state and the active state. The activation of ERM proteins *in vivo* involves binding to PI(4,5)P_2 and phosphorylation of a conserved threonine residue in the C-ERMAD (Thr567 in human ezrin sequence numbering). The activation dissociates the FERM domain from the C-ERMAD. Upon activation, the ERM proteins functions as a cross-linker by binding to (A) cytoplasmic extensions of membrane proteins (such as ICAM, CD43, CD44, NEP) and (B) scaffolding proteins such as EBP50, which then bind to membrane proteins.

2.3. Rho family small GTPases

The Ras superfamily of small GTPase proteins, including the Rho GTPase family, are molecular switches that control many cellular proteins and functions [97]. The members of this superfamily have a slow intrinsic GTPase activity and hence their nucleotide state is largely controlled by activating proteins (GAPs), nucleotide exchange factors (GEFs) and nucleotide dissociation inhibitors (GDIs). The state of the switch is determined by the bound nucleotide with the "on state" corresponding to bound GTP. The nucleotide controls the structure of two regions called switch I and switch II. In the GTP-bound on state, these two regions maintain a common structure which forms the interface with binding proteins controlled by the small GTPase [98]. Thus these small GTPases directly activate the first downstream partners by binding through the switch I/switch II surface.

Small GTPases, including the Rho family, are membrane associated proteins [99]. The interaction between the small GTPase and the membrane is usually mediated by multiple weak interactions. These include single or double covalent lipid modification of the GTPase (palmitoylation, myristoylation and/or prenylation) and polybasic or polyproline regions that bind to negatively charged lipids [99]. The activation of Rho GTPases enhances this membrane binding [99]. The Rho GTPases cycle between the cytosol, where they are maintained in an inactive Rho-GDP form by binding to the nucleotide dissociation inhibitor RhoGDI, and their site of action, the target membranes, where they are in an active, Rho.GTP membrane-bound state [100].

The Rho family of small GTPases (Rho, Rac and Cdc42) control membrane structure, dynamics and vesicle trafficking by modulating the actin cytoskeleton [101,102]. They act at various points in the membrane:cytoskeletal interface including: control of actin assembly/nucleation (DRFs, WAVE/WASP, cofilin and profilin); proteins that couple membranes to F-actin (ERM, EBP50, NHERF); proteins that produce PI(4,5)P_2 (PIP5K); the NADPH oxidase; and Rho kinase (ROCK). The CLIC proteins have also been linked to Rho GTPases [12,22,34,103].

The control of the membrane:actin cytoskeletal interface contains several feedback routes. Rho GTPases activate PIP5K, which produces the phospholipid PI(4,5)P_2. This, in turn, binds to several proteins activated by Rho GTPases including cofilin, profilin and ERM. Active ERM binds to RhoGDI releasing Rho so it can bind GTP, thus, keeping Rho GTPases in an active state, producing a positive feedback effect [104]. Paradoxically, there also appears to be a negative feedback loop between Rho GTPases and ERM [105].

2.4. NADPH oxidase

NADPH (nicotinamide adenine dinucleotide phosphate) oxidase is the reactive oxygen species (ROS) generating oxidase. The founding member NOX2 is predominantly expressed in phagocytes, such as macrophages. In the resting state it is composed of the integral membrane subunits gp91phox and p22phox and the soluble, cytosolic subunits p67phox, p47phox, p40phox and Rac2, a Rho GTPase. Upon activation by phagocytosing invading pathogens, the soluble subunits of the phox complex, including Rac2, are recruited to phagosomal membranes where they assemble with the membrane-bound subunits gp91phox and p22phox to form an active oxidase complex [106]. Both p40phox and p47phox proteins contain PX domains that target specific membranes by binding the phospholipids phosphatidylinositol 3-phosphate (PI(3)P) [107] and phosphatidylinositol-3,4-bisphosphate (PI(3,4)P_2) [108], respectively.

A crucial step in the assembly and the activation of the NADPH oxidase is the binding of Rac2 to p67phox. The cytosolic inactive Rac.GDP-GDI complex dissociates upon activation in a strictly GTP dependent manner, resulting in direct binding of active Rac.GTP to p67phox via the all α helical structural tetratrico-peptide repeat motif [109]. Recent evidence also shows that a low ROS environment

favours the activation of Rho (RhoA and RhoC). Therefore, ROS helps mediate the reciprocal regulation between Rac and Rho. Aberrant Rac and Rho activity has been linked to pathological conditions such as cancer [110].

2.5. PI(4,5)P_2 & PIP5K

Phosphatidylinositol-4-phosphate-5-kinase (PIP5K or PIPK5) is a ~500 residue protein that catalyses the phosphorylation of phosphatidylinositol-4-phosphate to PI(4,5)P_2 (phosphatidylinositol 4,5-bisphosphate). PI(4,5)P_2 controls the actin cytoskeleton by binding to cofilin, profilin and ERM proteins as well as other proteins involved in membrane:cytoskeleton interactions [111]. PI(4,5)P_2 is involved in the activation of ERM, as discussed above. PIP5K is regulated by Rac and Rho, two members of the Rho family of small GTPases. Active PIP5K is recruited to the membrane where it produces PI(4,5)P_2. This, in turn, maintains the active Rho-GTP state, providing positive feedback, as described above.

Both PIP5K and PIP4K are structurally related membrane associated proteins [112]. They are dimers with a flat, basic surface that docks onto a membrane. They have two binding pockets on the lipid surface so that substrates only need to move ~1 Å out of the plane of the membrane during catalysis. The proteins are stable, soluble proteins that only bind weakly to membranes.

PIP5K is a mediator of phagocytosis [113]. Fine control over the formation of a phagocytic cup is achieved by control of the spatial location of phosphatidyl inositol phosphates: PI(4,5)P_2 and PI(3,4,5)P_3. The nature of the phosphatidyl inositol phosphate determines which proteins are bound to the phagosome membrane, thus, these phospholipids orchestrate the phagocytic process.

The unstructured C-terminal region of the type Iβ PIP5K has been shown to bind EBP50 *in vitro*, using purified proteins, and in HL-60 cells [114]. Additionally, yeast two hybrid screens suggest that there may be an interaction between the unstructured C-terminal region of the type 1β PIP5K and both CLIC1 and CLIC4 (http://www.signaling-gateway.org, last accessed March 2013).

3. Setting the scene

3.1. Plasma membrane

3.1.1. Ezrin, microvilli & vesicular trafficking

Ezrin was originally discovered as a component of microvilli from intestinal epithelial cell brush borders [115]. It is associated with plasma membrane regions containing densely packed actin filaments, cellular structures such as: microvilli, ruffling membranes, cleavage furrows, cell-cell and cell-substrate adhesion sites [63,116].

Ezrin knockout mice do not survive >1.5 weeks after birth, hence conditional knockout [117,118] and knockdown [119] mice have been generated. Ezrin is the only ERM protein detected in epithelial cells of the developing intestine. Consequently, germline *Ezrin*$^{-/-}$ mice displayed abnormal intestinal microvillus morphogenesis, however, ezrin was not required for microvillus formation or epithelia polarisation [117]. Ezrin may play an essential role in configuring the apical terminal web region that provides a platform for anchoring the brush border microvilli and apical cell-cell junctions [117]. A similar phenotype was observed when the ezrin gene deletion was targeted only to adult intestinal epithelia [118]. This implies that ezrin is essential for both morphogenesis and homeostasis, particularly in the apical junctional region, perhaps by controlling cortical architecture and receptor distribution and activation.

The ezrin knockdown mice show severe growth retardation with only 7% surviving to adulthood [119]. These survivors showed no gross histological abnormalities with normal intestinal epithelial cells, well-formed intestinal villi as well as normal microvillar structures. The surviving ezrin knockdown mice suffered from severe achlorhydria (loss of gastric acid secretion) [119]. These mice have <5% of normal ezrin protein levels in their stomachs without compensatory upregulation of radixin or moesin. In the wild type (WT) mice, the parietal cells of the stomach are characterised by a meandering invagination of the apical surface called the secretory canaliculus [119]. This surface bears the microvilli. Underneath this, the parietal cell cytoplasm is densely packed with tubulovesicles. The parietal cells in the ezrin knockdown mice contain the tubulovesicles but not the invaginated secretory canaliculus.

Histamine stimulation of parietal cells from WT mice resulted in increased invaginations in the canaliculus, with a concomitant decrease in the density of underlying tubulovesicles [119]. In contrast, histamine stimulation of ezrin knockdown parietal cells did not result in the formation or expansion of the canalicular apical membranes and the cytoplasm remained densely packed with tubulovesicles [119].

These observations link the structure of the microvillar apical membrane with cytoplasmic tubular vesicles. This suggests that the defect caused by the reduction in ezrin may be related to the trafficking and fusion of the underlying tubulovesicles to the apical plasma membrane where they would increase the surface area and hence result in formation/expansion of the canalicular apical membrane.

3.1.2. CLIC5, ERM, the actin cytoskeleton & microvilli

The first indication of an association between CLIC proteins and ERM came with the discovery of CLIC5 in pull-down assays from extracts of placental microvilli using the ezrin C-ERMAD domain as bait [2]. Additionally, CLIC5 and ezrin colocalised to the apical microvilli of JEG-3 choriocarcinoma cells [120]. A complicating factor in the pull-down assay was that portions of the actin cytoskeleton were also pulled down, including actin, gelsolin, IQGAP1, α-actinin and ezrin itself. However, using latrunculin as an actin polymerisation inhibitor, subsequent pull-down studies confirmed the binding of ezrin to GST-CLIC5, with a reduced level of actin and α-actinin binding. The authors suggested that CLIC5 binds directly to ezrin, but not to the C-ERMAD [120].

3.1.3. CLIC5, radixin & stereocilia

The naturally occurring Jitterbug mouse has a mutated CLIC5 gene (*Clic5*$^{-/-}$) resulting in the absence of detectable CLIC5 protein expression [121]. These mice have progressive deafness and vestibular dysfunction (loss of balance) associated with hair cell stereocilia degeneration. The stereocilia of the inner ear are microvillar structures that protrude from the apical surface of the hair cells and are responsible for hearing and balance. Like other microvilli, the stereocilia are based on bundles of cross-linked actin filaments that are anchored to a terminal web and the membrane at the tip of the villi.

In normal mice, CLIC5 is highly expressed and colocalised with radixin in both cochlear and vestibular hair cell stereocilia [121]. In hair cells of the chicken utricle, CLIC5 is expressed with radixin at 1:1 molar ratio [121]. CLIC5 is also localised to the microvilli-covered apical surfaces of interdental cells and columnar cells of Kolliker's organ (a transient structure in the developing mammalian cochlea). In contrast, CLIC5 could not be detected in the inner ear of *Clic5*$^{-/-}$ mice and radixin expression was diminished [121]. Interestingly, mutations in the radixin gene are associated with nonsyndromic hearing loss in humans [122].

Ezrin, like radixin, is also present in the hair cell stereocilia [123,124]. In young *Rdx*$^{-/-}$ mice, both cochlear and vestibular stereocilia develop normally. However as the mice reach adulthood, cochlear stereocilia degenerated (as per the *Clic5*$^{-/-}$ mice), indicating that radixin is required for maintenance of cochlear stereocilia. This suggests that ezrin compensates for radixin during stereocilial development and vestibular stereocilial maintenance [123]. The fact that *jitterbug* mice exhibit both inner ear and vestibular dysfunction suggests that CLIC5 interacts with both radixin and ezrin to maintain healthy hair cell stereocilia.

3.1.4. CLIC5, ezrin & the maintenance of the filtration barrier in the kidney

The podocyte cells of renal glomeruli create the filtration barrier that prevents high molecular weight components of blood from diffusing into urine. Each podocyte creates a comb-like structure comprising long, thin, actin-filled extensions of the cytoplasm called foot processes [125]. The filtration barrier is formed by the specialised "zipper-like" cell-cell junctions between adjacent foot processes [126]. Podocalyxin is a podocyte apical membrane sialoglycoprotein which binds ezrin and the scaffolding protein NHERF1. The Rho family GTPase Cdc42 is critical for the maintenance of the filtration barrier structure [127]. In cultured MDCK cells ezrin recruits RhoGDI, facilitating the activation of Rho GTPases [128].

CLIC5 and ezrin localise to the podocyte foot processes and are essential for healthy kidney function [129,130]. CLIC5 is highly expressed in podocytes and endothelial cells of glomeruli [131], while ezrin connects the cytoplasmic tail of podocalyxin to the actin cytoskeleton [132,133]. CLIC5 colocalises with ezrin and podocalyxin in glomeruli and coimmunoprecipitates with podocalyxin from a glomerular lysate.

In addition to the defects in hearing and balance discussed above, *Clic5*$^{-/-}$ *jitterbug* mice have proteinuria [130] with broadened podocytes and vacuolisation of their glomerular endothelial cells [129]. Western blots of glomerular lysates from *jitterbug* mice show that ezrin expression level is reduced compared to wild type. Additionally, the colocalisation of ezrin and podocalyxin in podocytes is reduced in *jitterbug* glomeruli, breaking the link between podocalyxin and actin. Dissociation of podocalyxin from the actin cytoskeleton is associated with the loss of podocyte structural integrity [133]. Hence, CLIC5 plays a role in maintaining both structure and function of the podocyte by facilitating the interaction between ezrin and podocalyxin.

3.1.5. CLIC4, ezrin & the apical microvilli of the occular retinal pigment epithelium

The retinal pigment epithelium (RPE) of the eye is a layer of pigmented cells sandwiched between the photoreceptors and the choriocapillaries [134]. This layer serves as a blood-retina barrier. The apical side of the RPE faces the photoreceptors and develops long microvilli that interdigitate into and tightly embrace the light sensing outer segments of the photoreceptors. The RPE phagocytoses the decayed disks of the photoreceptors.

Ezrin is important for the maintenance of the RPE microvilli. Reducing the expression of ezrin in primary cultures of rat RPE decreased the length and number of apical microvilli and the elaborate basal infoldings typical of these cells [135]. Further, *Ezrin*$^{-/-}$ mice bear similar morphological changes in RPE [136].

Like Ezrin, CLIC4 is also enriched in the apical microvilli of RPE [134]. Using *in vivo* retinal transfection, the silencing of CLIC4 in rat RPE resulted in changes similar to that of ezrin knockdown or gene deletion. They displayed loss of apical microvilli and basal infoldings, reduced retinal adhesion and epithelial-mesenchymal transition [134]. CLIC4 suppression also resulted in severe dysplasia in adjacent neural retinas, suggesting it is critical for retinal epithelial morphogenesis [134]. Unexpectedly, high level of the water channel, aquaporin 1, appeared at the apical surfaces of cells in which CLIC4 expression was suppressed.

3.2. Intracellular membranes & tubulogenesis

Tubulogenesis is a fundamental cellular process needed for the development of tubules in organs such as kidneys, lungs and blood vessels. Tubules arise from the hollowing of polarised epithelial or endothelial cells and involve extensive cytoskeletal reorganisation and membrane trafficking [137,138]. Not surprisingly, ERM proteins and Rho GTPases are critical regulators of tubulogenesis [139].

3.2.1. Exc phenotype in C. elegans: CLIC-like proteins, ERM, Rho & cytoskeleton

In the nematode, *C. elegans*, the excretory cell forms an "H"-like structure that runs the length of the worm. Inside this cell, there is a tubular, apical membrane-bound compartment into which wastes are excreted. The worm excretory system has been used as a model for the kidney.

In 1999, a series of mutants was described where this apical membrane domain, the excretory canal, was defective, usually forming cysts [140]. Nine *exc* mutants (*exc-1* through *exc-9*) and mutations of several other genes can cause this phenotype. Some of the proteins now known to cause this mutation include: Sma-1–β spectrin [140]; Exc-5–FGD1, a GEF for Cdc42 [141–143]; Exc-7–ELAV, a splicing factor that binds mRNA for Sma-1 and Exc-3 [144,145]; and Exc-9–Zn-binding LIM domain protein [146].

The discovery that Exc-4 was a member of the CLIC protein family was a major advance in understanding CLIC function [17,18]. Unlike mammalian CLICs, Exc-4 does not have a detectible cytosolic component but lines the apical tubular membrane in the excretory cell and is completely membrane associated. The key determinant for membrane binding is helix h1 [16], which corresponds to the putative TM domain in human CLICs [17,18]. Disruption of Exc-4 results in a cystic enlargement of the interior lumen of the canal of the excretory cell. Despite this, the precise mechanism by which *exc4* mutations maintain the integrity of the excretory canal is not known.

Some of the other *exc* mutations have functions that might intersect with that of Exc-4 as they are involved in membrane turnover or remodelling. For example, the GEF Exc-5 regulates the Rho GTPases, Cdc42 and Rac, in a manner that controls endosome recycling [141]. When Exc-5 is disrupted, early endosomes concentrate near the apical membrane while recycling endosomes are depleted. Conversely, the over-expression of Exc-5 results in enrichment of recycling endosomes. It is thought that endosome recycling is important for maintaining the integrity of the luminal membrane.

C. elegans has a single ERM protein, ERM-1 which has an essential role in lumen morphogenesis [147,148]. Disruption of the *erm-1* gene results in a cystic luminal phenotypes like that in the Exc mutants. ERM-1 interacts with the cytoskeletal proteins, β spectrin (Sma-1) and actin 5, in maintaining the luminal membrane [147]. Further, ERM-1 recruits the water channel, AQP-8, to the excretory cell lumen and this channel is important in directly controlling fluid pressure across the membrane [149].

3.2.2. CLIC4 & tubulogenesis

In tubulogenesis, lumen formation and expansion require apical membrane biogenesis and serial intracellular vacuole and vesicle fusion events [150,151]. Compared to the near neutral cytosolic pH, vesicles are acidic with a varying pH that is regulated by a number of ion channels and transporters such as the vacuolar H^+ ATPase (v-ATPase). v-ATPase utilizes the energy of ATP hydrolysis to translocate protons across membranes resulting in a lowered luminal pH and an inside-positive transmembrane potential. Thus, sustaining v-ATPase activity requires a parallel counterion transport to compensate the charge build up. Chloride ion regulation through chloride channels and transporters including the CLICs are suggested to play an important part in providing these counterions [152].

Supporting a role for CLICs in counterion regulation, *Clic4*$^{-/-}$ mice that display defective angiogenesis also have impaired vacuolar acidification [153]. In tubulating endothelial cells, the average pH of large vacuoles is about 0.3 pH units higher in *Clic4*$^{-/-}$ mice. There is no pH difference in the small vesicles that resemble lysosomes. Both the CLIC channel blocker IAA94 and the v-ATPase inhibitor bafilomycin A1 caused impairment in WT endothelial cell vacuolar acidification, supporting the involvement of both proteins in this process [153]. The outcome of impaired angiogenesis is that *Clic4*$^{-/-}$ mice have reduced arterial collaterals in skeletal muscle and brain [154], a process

that may also account for the high rate of stillbirths seen in these mice.

4. Action!

Implicit in the scenarios above is the dynamic nature of the cellular processes involving CLIC and ERM. Below, we examine cellular processes where the dynamics are explicit.

4.1. CLIC4, NHERF2, RhoA & translocation to the plasma membrane

A dramatic movement of CLIC4 to the plasma membrane was observed in NIE-115 neuroblastoma cells on stimulation with either lysophosphatidic acid (LPA), thrombin receptor activating peptide or sphingosine-1-phosphate [22]. This was observed statically, using immunofluorescence and endogenous CLIC4, and dynamically, using live cell imaging in cells expressing GFP-CLIC4. CLIC4 movement to the plasma membrane was dependent on $G\alpha_{13}$-mediated RhoA activation and F-actin integrity, but not Rho kinase activity [22] and was coincident with recruitment of NHERF2 to same sites. As NHERF2 is an ERM binding scaffolding protein, this suggests the involvement of ERMs in this process.

The putative enzymatic site for CLIC4 centres on Cys35, which is equivalent to Cys24 in CLIC1 (Fig. 1A & B). Any mutations around this site blocked CLIC4 membrane recruitment and prevented NHERF2 colocalisation [22]. This suggests that CLIC4 may possess a Cys-dependent transferase activity which is linked to its translocation to the plasma membrane sites.

4.2. CLIC1, amyloid & translocation to the plasma membrane

In microglial BV2 cells, the addition of amyloid Aβ peptide produced a translocation of cytoplasmic CLIC1 to the plasma membrane, presumably to the site of amyloid particle ingestion [27]. The movement of CLIC1 was associated with increased CLIC1 ion channel activity in the plasma membrane [155], as well as NADPH oxidase mediated production of ROS [27].

4.3. CLIC1, ERM, Rho, Rac & phagocytosis

CLIC1 is highly expressed in macrophages, which are key cells in innate and adaptive immunity. In resting macrophages, CLIC1 appears as punctate structures with a dense perinuclear distribution [34] (Fig. 3). Although this distribution is similar to known vesicles, the CLIC1 structures do not co-localise with markers for early endosomes (EEA1), transferrin-positive endosomes, late endosomes (LBPA), lysosomes (LAMP1) or recycling endosomes (Rab11) [34].

Macrophages ingest pathogens, foreign particulates or apoptotic cells by phagocytosis to form phagosomes. During the course of phagocytosis, phagosomes mature progressively by fusion with acidic early and late endosomes, as well as lysosomes, resulting in progressive phagosomal acidification [156,157]. The process of phagosome maturation is in part dependent on Rho GTPases and ERM proteins, both of which are regulators of the reorganisation of the actin cytoskeleton [94].

Phagocytosis of serum opsonised zymosan particles by macrophages results in complex, rapidly occurring changes to plasma and intracellular membranes. On initiation of this process, the small GTPases, Rac2 and RhoA, translocate to the phagosomal membrane [94], which is followed rapidly by translocation of CLIC1 and ERM [34] (Fig. 3D-L). *Clic1*$^{-/-}$ mice display a small but consistent defect in phagosomal acidification of about 0.2 pH units [34]. This pH difference could be reproduced in WT but not *Clic1*$^{-/-}$ macrophages using the CLIC ion channel blocker IAA94. The pH increase in knockout cells was not due to altered phagosomal-lysosomal fusion. This data bears strong similarity to the changes in vesicle pH described in CLIC4 $^{-/-}$ endothelial cells during tube formation [153].

Consistent with an effect on phagosomal pH, *Clic1*$^{-/-}$ macrophages also show impaired phagosomal proteolysis and attenuated ROS production. Further, *Clic1*$^{-/-}$ mice were almost completely protected from arthritis development in the K/BxN serum transfer arthritis, a disease model of chronic inflammation that is dependent on innate immune cells like macrophages [158].

4.4. CLIC3 & the sorting/recycling of intracellular vesicles

Endosome trafficking is important in the recycling of cell surface receptors/proteins. Receptor-ligand interaction often results in internalisation of the complex into endosomes which may then either fuse with lysosomes, resulting in the degradation of their cargo, or be recycled to the plasma membrane. Studies in an ovarian cancer cell line show that the small GTPase, Rab25, is important in directing endosomes containing active α5β1 integrin to lysosomes [159]. Rab25 also upregulates CLIC3 which then colocalises with active α5β1 on late endosomes/lysosomes. Both CLIC3 and the acidification of the late endosome/lysosome are essential for the recycling of the active α5β1 integrin to the plasma membrane.

Similar findings were obtained by silencing a sialidase, NEU3, in renal carcinoma cells [160]. NEU3 regulates β1 integrin trafficking by controlling recycling. Silencing NEU3 upregulated Rab25, directing internalised integrins to lysosomes, and downregulated CLIC3, blocking recycling to the plasma membrane [160].

Studies using a peptide, CLT1, that binds to tumour interstitial spaces showed that when the peptide aggregated with fibronectin, it promoted the translocation of CLIC1 to the endothelial cell surface via ligation to integrin α5β3 [161]. The internalisation of this complex led to tumour cell death. When added to bladder tumour cell lines [162], this peptide induced autophagic tumour cell death that was dependent on the integrin α5β1 and CLIC3. These authors propose that the internalisation of the CLT1-fibronectin complex results in lysosomal dysfunction which triggers autophagy [162].

Pathogens such as *Listeria monocytogenes* can survive in macrophages after being phagocytosed. The killing of these pathogens requires bacteria to be opsonised by the complement product, C3b [163]. This killing activity is effected by the fusion of phagosomes with lysosomes. CLIC3 was necessary for phagosome-lysosome fusion and is responsible for an increase in chloride ion concentration in the phagosome from ~30 mM to ~100 mM and was independent of phagosomal acidification [163].

5. Is there a smoking gun?

A common thread in all the observations above is that CLIC and ERM proteins are often involved in membrane remodelling processes that also involve the actin cytoskeleton and small, usually Rho, GTPases. Some of these processes involve specialised plasma membrane structures such as microvilli, stereocilia and podocytes, while others involve the formation of membrane vesicles (phagocytosis) or vesicle trafficking (phagosome-lysosome fusion, endosome recycling). In some of the processes that involve specialised plasma membrane structures, the underlying mechanism may involve the trafficking of vesicles from the underlying cytoplasm to the plasma membrane.

This suggests that CLIC and ERM proteins may interact, directly or indirectly, to facilitate actin-mediated membrane remodelling. Despite this, there is no compelling evidence for a strong, direct interaction between CLIC and ERM proteins, or between these proteins and Rho GTPases or PIP5K. In part, the lack of direct evidence may be due to the nature of the interactions involved.

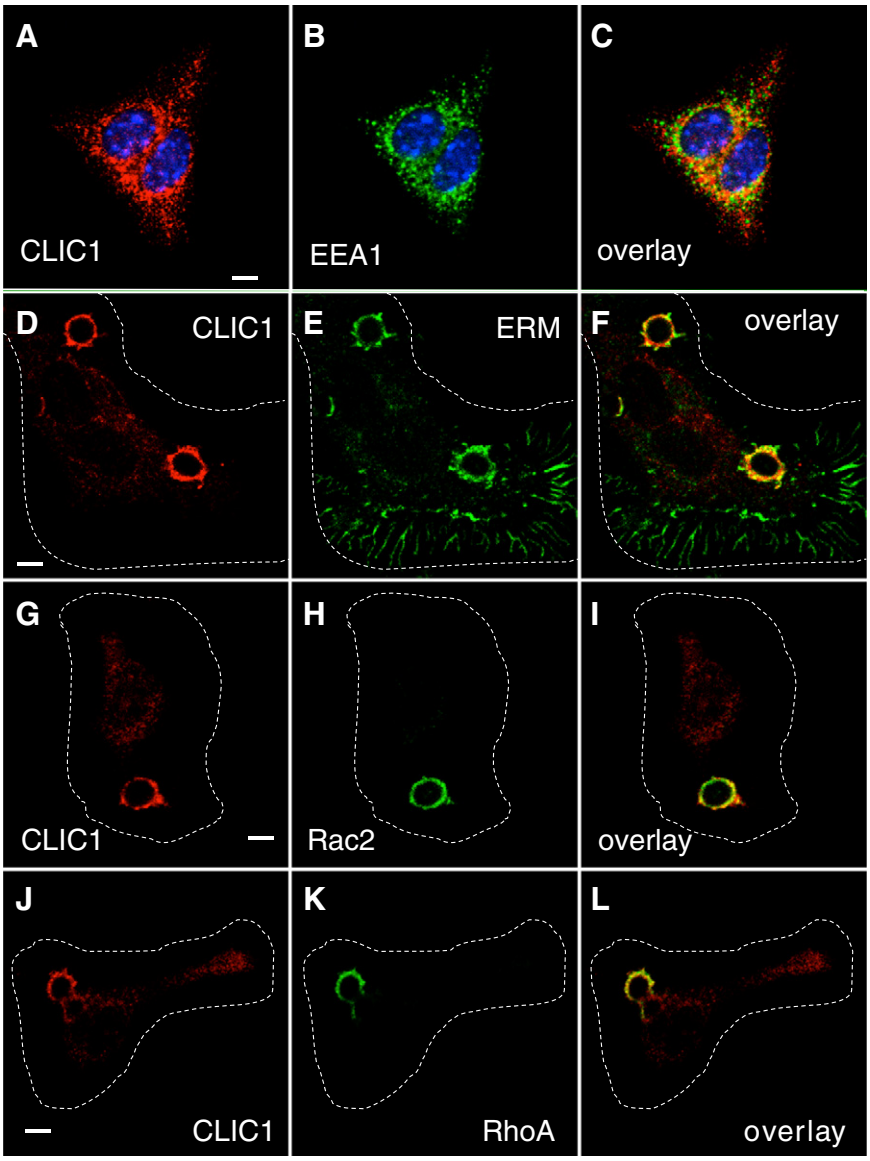

Fig. 3. Subcellular distributions of CLIC1, ERM & RhoGTPases during phagocytosis. Protein subcellular localisation in macrophages as shown by fluorescence confocal microscopy. A: In resting macrophages, CLIC1 appears in confocal immunostaining images as punctate, unidentified vesicle-like cytoplasmic structures with a dense perinuclear distribution. B: These are similar to the early endosome marker EEA1. C: In the periphery of the cell where the cytoplasm is thin, it is clear that CLIC1-containng structures do not colocalise with the EEA1 positive early endosomes. When macrophages ingest serum opsonized zymosan particles (panels D to L), CLIC1 translocates to phagosomal membrane (D, G, J) where it appears to colocalise with ERM (E, F), Rac2 (H, I) and RhoA (K, L). The nucleus is shown in blue (A, B, C) after staining with To-Pro-3. Bar = 5 μm.

5.1. Multiple weak interactions and coincidence signalling

Many proteins at the membrane:cytoskeletal interface interact weakly with each other and with membranes. However, when multiple weak interactions involve a common target, such as a membrane or multiple sites on a single protein, they can produce strong overall binding [164].

The prevalence of such multiple weak interactions at the membrane has led to the concept of coincidence signalling, where the presence of two or more membrane bound species (proteins or lipids) is needed to produce a high affinity complex with an effector protein [165]. This may explain aspects of Rho signalling and the regulation of Rho by GEFs and PIP5K by Rho.

The use of multiple weak interactions in coincidence signalling has many advantages. It allows fine control, where breaking a single weak interaction reduces the affinity of the whole signalling complex. It allows for signal integration, where multiple inputs are needed for signal transmission. This allows for circuit expansion, where common signalling components can be rearranged and reused to create new networks.

5.2. Do Rho GTPases activate ERM by direct binding?

A clue to how the ERM system is activated by Rho GTPases comes from studies of the sorting nexins, SNX17, SNX27 and SNX31, a family of phox-homology domain containing proteins that are involved in vesicle trafficking [166–168]. The SNX proteins contain a FERM-like domain where FERM subdomain F1 is homologous to a Ras-association domain. Only active GTP-bound Ras proteins bind to the SNX FERM domain, however, this binding is weak (~20 μM) [166,167].

Recently, the crystal structure of the complex between the FERM domain of KRIT1 and the small GTPase Rap1 has been reported [169]. This structure confirms the predictions based on the sorting nexins. The interface includes the switch I and switch II regions making the interaction sensitive to the presence of GTP. Thus, it is likely that many FERM domain proteins are effectors of small GTPases.

It is possible that active Rho GTPases and ERM proteins make similar, weak, direct complexes in solution. In order to form a high affinity complex, the missing component may be the membrane, which would have multiple attachment sites for ERM (via cytoplasmic domains of integral membrane proteins and charged phospholipids) and for the active Rho.GTP complex.

5.3. Unstructured cytoplasmic domains: "string binding"

Integral membrane proteins often have unstructured cytoplasmic domains or "strings". These strings acquire structure when they meet their target binding protein. The ERM FERM domain binds such cytoplasmic string domains of membrane proteins. The scaffolding proteins including EBP50 and NHERF do likewise, extending the number of strings attached to the ERM:scaffolding protein complex. This increases the number of interactions between membrane components and the scaffolding complexes, which is consistent with the notion of multiple weak interactions and coincidence signalling.

CLIC proteins may also bind extended string domains. One unusual feature of the CLIC protein structures is the presence of an open slot that includes the putative active site [13,20,22,30,32,170]. This slot could accommodate an extended polypeptide chain and, indeed, normally unstructured regions of neighbouring CLIC molecules lie in this slot so as to form crystal contacts [30,32]. It is possible that the targeting of CLIC proteins to membrane surfaces either with or without CLIC enzymatic activity, involves binding to cytoplasmic, unstructured regions of membrane proteins. This might explain why mutagenesis of the CLIC4 active site prevented its membrane localisation after GPCR stimulation and subsequent Rho activation [22].

5.4. Are CLIC proteins soluble, cytoplasmic proteins or are they components of intracellular vesicles?

The nature of the cytoplasmic pool of CLIC proteins is unresolved. In *C. elegans*, there appears to be little free cytoplasmic CLIC (Exc-4), with the majority of the protein residing on the apical luminal membranes. Mammalian CLIC proteins have no signal sequence and are synthesised in the cytoplasm. Most of the mammalian CLIC protein resides in the cytoplasm, however, it is not clear as to whether this is as a soluble protein, or bound to vesicles. The immunofluorescence imaging studies of CLIC1 in Panc 1 (human pancreatic carcinoma epithelial-like) cells [171], platelets [103] and macrophages [34] (Fig. 3) all show punctate staining that is densely packed in the perinuclear region. This is reminiscent of endosomes and other intracellular vesicles. In contrast, studies using overexpressed CLIC-GFP fusion proteins display a diffuse staining pattern, for example, CLIC1-eGFP in BV2 cells [27] and GFP-CLIC4 in NIE-115 neuroblastoma cells [22]. Determining the nature of the cytoplasmic pools of CLIC proteins (vesicle bound *versus* soluble) will help advance our understanding of the molecular mechanisms involved in CLIC biology.

5.5. Do CLIC proteins function as ion channels in intracellular vesicles?

The most direct evidence to date of physiologically relevant ion channel activity comes from our studies on macrophage phagocytosis where *Clic1*$^{-/-}$ phagosomes were defective in acidification [34]. The simplest interpretation of the difference between phagosomal pH when comparing *Clic1*$^{-/-}$ to WT is that CLIC1 acts as an ion channel, compensating for the change in membrane potential as the luminal

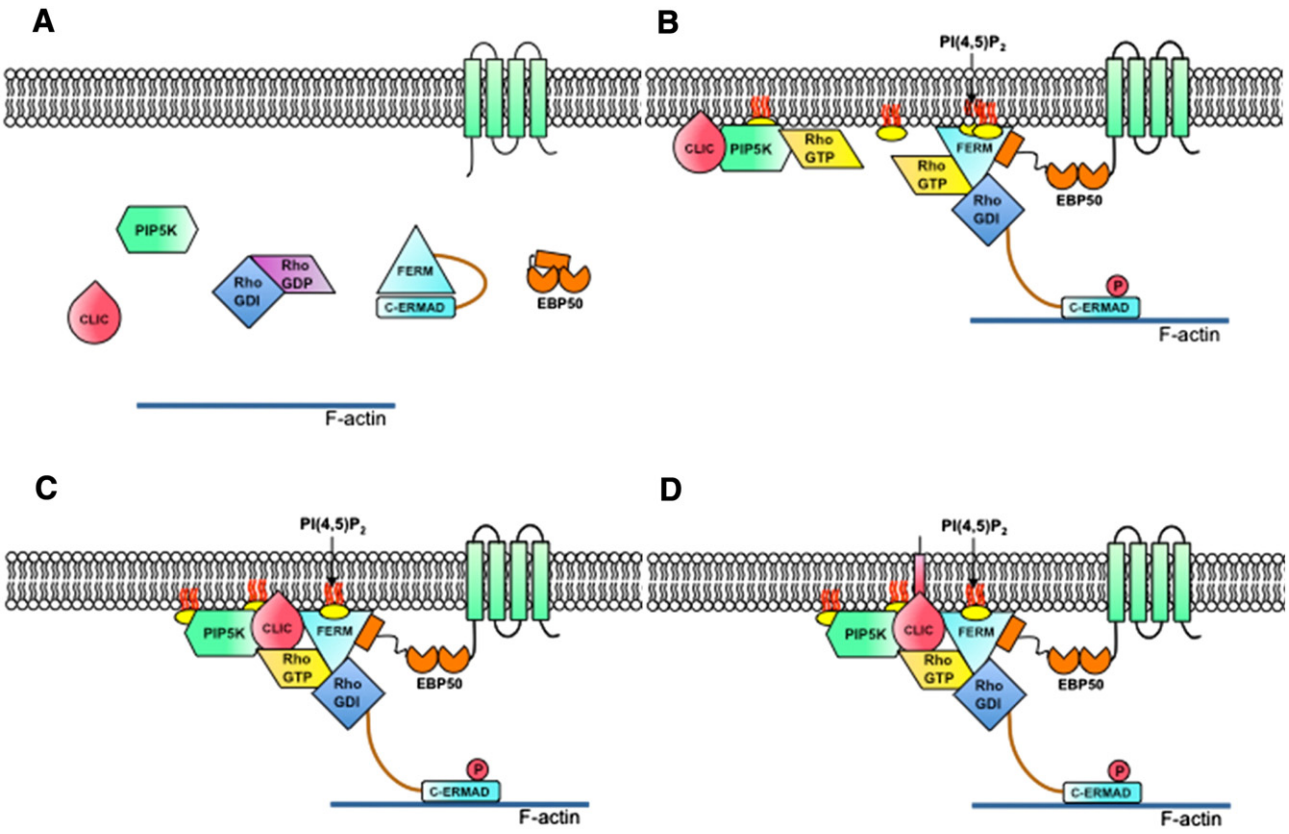

Fig. 4. *Speculative model for assembly of CLIC-ERM-Rho complexes on membranes*. A: The dormant state prior to signalling. Rho.GDP is bound to RhoGDI, and hence inactive. ERM and EBP50 are in their inactive states and CLIC proteins are in the cytoplasm. B, C & D. Three possible scenarios for the active, signalling state. B: Rho.GTP binds the membrane, recruits PIP5K and CLIC. Active PIP5K produces $PI(4,5)P_2$. Rho.GTP also binds ERM, helping in its activation via a kinase. EBP50 is also activated. ERM binds to $PI(4,5)P_2$ on the membrane and EBP50, which binds to cytoplasmic domains of integral membrane proteins. ERM binds to RhoGDI, ensuring continued activity of Rho. ERM C-ERMAD recruits F-actin. C: This is a variant of B where CLIC binds to ERM as well as PIP5K resulting in the formation of a large complex on the membrane. D: is a variant on C where CLIC integrates into the membrane producing and integral membrane CLIC protein which may have ion channel activity.

pH decreases. Further, these mice displayed no defect in phagosome-endosome fusion that might otherwise account for the altered pH. This data is consistent with the acidification defect in the large vacuoles of *Clic4*$^{-/-}$ endothelial cells [153] and the lowered intra-endosomal chloride concentration in *Clic3*$^{-/-}$ bone marrow derived macrophages [163]. However, without direct mechanistic information for how these pH/chloride ion differences occur, it is impossible to be certain that a direct ion channel function of the CLIC proteins is responsible.

5.6. A speculative model for the assembly of a membrane-bound complex including CLIC, ERM, RhoGTPase

Drawing on all the data above, we propose speculative models for what is occurring at the membrane surface involving CLIC proteins, ERM and Rho GTPases. Initially, the membrane is free of CLIC, ERM, Rho and the scaffolding proteins (Fig. 4A). Rho GTPases may form complexes with RhoGDI, maintaining them in the inactive Rho.GDP state. A cellular signal initially activates a Rho GTPase, moving it from its cytoplasmic Rho.GDP state to its membrane-bound Rho.GTP state. This activates PIP5K, producing PI(4,5)P_2 locally in the membrane (Fig. 4 B, C & D). Active RhoGTP binds ERM, which is phosphorylated by a kinase (either ROCK or another kinase). ERM adopts its active configuration and binds to the membrane via PI(4,5)P_2. It binds to actin filaments with its free C-ERMAD. ERM recruits scaffolding proteins and together they bind the cytoplasmic tails of integral membrane proteins causing them to cluster.

The question is: where is CLIC in this process? Some experimental data shows that CLICs move to the membrane on Rho activation. Therefore, one possibility is that CLICs binds to membrane-associated PIP5K as suggested by the yeast two hybrid screens, by binding to the unstructured C-terminal domain of PIP5K (Fig. 4B). A second possibility is that the CLIC binds directly to ERM, potentially linking the ERM complex to PIP5K (Fig. 4C). It is not clear whether CLIC proteins bind to the membrane as part of a complex or whether they integrate into the membrane, possibly forming an ion channel (Fig. 4D). The cytoplasmic state of CLIC proteins are still unresolved. If CLICs are bound to perinuclear vesicles in quiescent cells, Rho activation may recruit CLIC-associated vesicles to the site of activation (Fig. 5).

6. Conclusion

The interface between the actin cytoskeleton and membranes is highly dynamic and tightly controlled. Many of the participating proteins interact with each other and the membrane via multiple weak interactions. These include interactions with PI(4,5)P_2 which is also a major signalling molecule. The resulting cellular signals are likely to occur via coincidence or integrated signalling. This allows maximal control and versatility in signalling. Many of the integral membrane

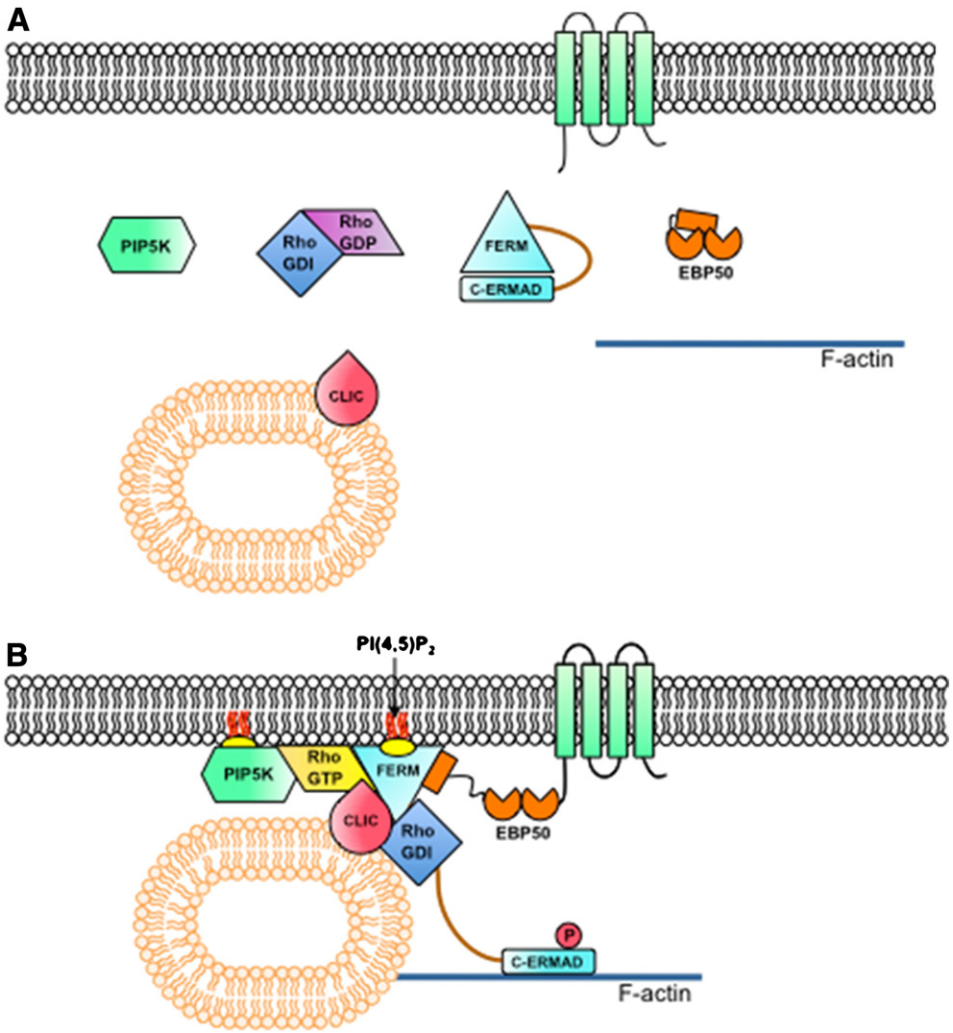

Fig. 5. *Speculative model where CLIC is involved in recruiting vesicles*. Cytoplasmic CLIC proteins may reside in/on intracellular vesicles. A: In the quiescent state, vesicle-bound CLIC lies in the perinuclear region. B: On Rho activation, proteins assemble at the site of activation and CLIC containing vesicles are transported to the site of activation.

proteins in these signalling complexes provide "strings" – unstructured peptides – that adopt a well-defined three dimensional structure only when they bind to target proteins such as the ERM FERM domain or the NHERF PDZ domains.

The CLIC proteins and the ERM family appear to be linked in maintaining the structure and dynamics of the membrane:actin cytoskeleton interface. They are controlled by the Rho GTPases and, in turn, control Rho, providing feedback stabilisation. CLIC and ERM appear to be important in determining the fate of intracellular vesicles — to fuse or not to fuse, to return to the plasma membrane or not. This may facilitate their control over plasma membrane structures such as microvilli, where they may be acting as a source of plasma membrane material rather than directly stabilising static structures. The molecular mechanism by which CLICs and ERMs cooperate in this is yet to be discovered.

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References

[1] T. Nishizawa, T. Nagao, T. Iwatsubo, J.G. Forte, T. Urushidani, Molecular cloning and characterization of a novel chloride intracellular channel-related protein, parchorin, expressed in water-secreting cells, J. Biol. Chem. 275 (2000) 11164–11173.
[2] M. Berryman, A. Bretscher, Identification of a novel member of the chloride intracellular channel gene family (CLIC5) that associates with the actin cytoskeleton of placental microvilli, Mol. Biol. Cell 11 (2000) 1509–1521.
[3] Z. Qian, D. Okuhara, M.K. Abe, M.R. Rosner, Molecular cloning and characterization of a mitogen-activated protein kinase-associated intracellular chloride channel, J. Biol. Chem. 274 (1999) 1621–1627.
[4] E. Fernandez-Salas, M. Sagar, C. Cheng, S.H. Yuspa, W.C. Weinberg, p53 and tumor necrosis factor alpha regulate the expression of a mitochondrial chloride channel protein, J. Biol. Chem. 274 (1999) 36488–36497.
[5] B.M. Tulk, J.C. Edwards, NCC27, a homolog of intracellular Cl− channel p64, is expressed in brush border of renal proximal tubule, Am. J. Physiol. 274 (1998) F1140–F1149.
[6] J.C. Edwards, B. Tulk, P.H. Schlesinger, Functional expression of p64, an intracellular chloride channel protein, J. Membr. Biol. 163 (1998) 119–127.
[7] S.M. Valenzuela, D.K. Martin, S.B. Por, J.M. Robbins, K. Warton, M.R. Bootcov, P.R. Schofield, T.J. Campbell, S.N. Breit, Molecular cloning and expression of a chloride ion channel of cell nuclei, J. Biol. Chem. 272 (1997) 12575–12582.
[8] R.R. Duncan, P.K. Westwood, A. Boyd, R.H. Ashley, Rat brain p64H1, expression of a new member of the p64 chloride channel protein family in endoplasmic reticulum, J. Biol. Chem. 272 (1997) 23880–23886.
[9] D. Landry, S. Sullivan, M. Nicolaides, C. Redhead, A. Edelman, M. Field, Q. al-Awqati, J. Edwards, Molecular cloning and characterization of p64, a chloride channel protein from kidney microsomes, J. Biol. Chem. 268 (1993) 14948–14955.
[10] D.W. Landry, M.H. Akabas, C. Redhead, A. Edelman, E.J. Cragoe Jr., Q. Al-Awqati, Purification and reconstitution of chloride channels from kidney and trachea, Science 244 (1989) 1469–1472.
[11] C.R. Redhead, A.E. Edelman, D. Brown, D.W. Landry, Q. al-Awqati, A ubiquitous 64-kDa protein is a component of a chloride channel of plasma and intracellular membranes, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 3716–3720.
[12] H. Singh, Two decades with dimorphic chloride intracellular channels (CLICs), FEBS Lett. 584 (2010) 2112–2121.
[13] D.R. Littler, S.J. Harrop, S.C. Goodchild, J.M. Phang, A.V. Mynott, L. Jiang, S.M. Valenzuela, M. Mazzanti, L.J. Brown, S.N. Breit, P.M. Curmi, The enigma of the CLIC proteins: ion channels, redox proteins, enzymes, scaffolding proteins? FEBS Lett. 584 (2010) 2093–2101.
[14] J.C. Edwards, C.R. Kahl, Chloride channels of intracellular membranes, FEBS Lett. 584 (2010) 2102–2111.
[15] R.H. Ashley, Challenging accepted ion channel biology: p64 and the CLIC family of putative intracellular anion channel proteins (Review), Mol. Membr. Biol. 20 (2003) 1–11.
[16] D.R. Littler, S.J. Harrop, L.J. Brown, G.J. Pankhurst, A.V. Mynott, P. Luciani, R.A. Mandyam, M. Mazzanti, S. Tanda, M.A. Berryman, S.N. Breit, P.M. Curmi, Comparison of vertebrate and invertebrate CLIC proteins: the crystal structures of *Caenorhabditis elegans* EXC-4 and *Drosophila melanogaster* DmCLIC, Proteins 71 (2008) 364–378.
[17] K.L. Berry, O. Hobert, Mapping functional domains of chloride intracellular channel (CLIC) proteins in vivo, J. Mol. Biol. 359 (2006) 1316–1333.
[18] K.L. Berry, H.E. Bulow, D.H. Hall, O. Hobert, A *C. elegans* CLIC-like protein required for intracellular tube formation and maintenance, Science 302 (2003) 2134–2137.
[19] N. King, M.J. Westbrook, S.L. Young, A. Kuo, M. Abedin, J. Chapman, S. Fairclough, U. Hellsten, Y. Isogai, I. Letunic, M. Marr, D. Pincus, N. Putnam, A. Rokas, K.J. Wright, R. Zuzow, W. Dirks, M. Good, D. Goodstein, D. Lemons, W. Li, J.B. Lyons, A. Morris, S. Nichols, D.J. Richter, A. Salamov, J.G. Sequencing, P. Bork, W.A. Lim, G. Manning, W.T. Miller, W. McGinnis, H. Shapiro, R. Tjian, I.V. Grigoriev, D. Rokhsar, The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans, Nature 451 (2008) 783–788.
[20] S.J. Harrop, M.Z. DeMaere, W.D. Fairlie, T. Reztsova, S.M. Valenzuela, M. Mazzanti, R. Tonini, M.R. Qiu, L. Jankova, K. Warton, A.R. Bauskin, W.M. Wu, S. Pankhurst, T.J. Campbell, S.N. Breit, P.M. Curmi, Crystal structure of a soluble form of the intracellular chloride ion channel CLIC1 (NCC27) at 1.4-A resolution, J. Biol. Chem. 276 (2001) 44993–45000.
[21] A. Dulhunty, P. Gage, S. Curtis, G. Chelvanayagam, P. Board, The glutathione transferase structural family includes a nuclear chloride channel and a ryanodine receptor calcium release channel modulator, J. Biol. Chem. 276 (2001) 3319–3323.
[22] B. Ponsioen, L. van Zeijl, M. Langeslag, M. Berryman, D. Littler, K. Jalink, W.H. Moolenaar, Spatiotemporal regulation of chloride intracellular channel protein CLIC4 by RhoA, Mol. Biol. Cell 20 (2009) 4664–4672.
[23] A.F. Dulhunty, R. Hewawasam, D. Liu, M.G. Casarotto, P.G. Board, Regulation of the cardiac muscle ryanodine receptor by glutathione transferases, Drug Metab. Rev. 43 (2011) 236–252.
[24] M. Malik, A. Shukla, P. Amin, W. Niedelman, J. Lee, K. Jividen, J.M. Phang, J. Ding, K.S. Suh, P.M. Curmi, S.H. Yuspa, S-nitrosylation regulates nuclear translocation of chloride intracellular channel protein CLIC4, J. Biol. Chem. 285 (2010) 23818–23828.
[25] S. Averaimo, R.H. Milton, M.R. Duchen, M. Mazzanti, Chloride intracellular channel 1 (CLIC1): sensor and effector during oxidative stress, FEBS Lett. 584 (2010) 2076–2084.
[26] S.C. Goodchild, M.W. Howell, N.M. Cordina, D.R. Littler, S.N. Breit, P.M. Curmi, L.J. Brown, Oxidation promotes insertion of the CLIC1 chloride intracellular channel into the membrane, Eur. Biophys. J. 39 (2009) 129–138.
[27] R.H. Milton, R. Abeti, S. Averaimo, S. DeBiasi, L. Vitellaro, L. Jiang, P.M. Curmi, S.N. Breit, M.R. Duchen, M. Mazzanti, CLIC1 function is required for beta-amyloid-induced generation of reactive oxygen species by microglia, J. Neurosci. 28 (2008) 11488–11499.
[28] C. Jalilian, E.M. Gallant, P.G. Board, A.F. Dulhunty, Redox potential and the response of cardiac ryanodine receptors to CLIC-2, a member of the glutathione S-transferase structural family, Antioxid. Redox Signal. 10 (2008) 1675–1686.
[29] H. Singh, R.H. Ashley, CLIC4 (p64H1) and its putative transmembrane domain form poorly selective, redox-regulated ion channels, Mol. Membr. Biol. 24 (2007) 41–52.
[30] B.A. Cromer, M.A. Gorman, G. Hansen, J.J. Adams, M. Coggan, D.R. Littler, L.J. Brown, M. Mazzanti, S.N. Breit, P.M. Curmi, A.F. Dulhunty, P.G. Board, M.W. Parker, Structure of the Janus protein human CLIC2, J. Mol. Biol. 374 (2007) 719–731.
[31] H. Singh, R.H. Ashley, Redox regulation of CLIC1 by cysteine residues associated with the putative channel pore, Biophys. J. 90 (2006) 1628–1638.
[32] D.R. Littler, N.N. Assaad, S.J. Harrop, L.J. Brown, G.J. Pankhurst, P. Luciani, M.I. Aguilar, M. Mazzanti, M.A. Berryman, S.N. Breit, P.M. Curmi, Crystal structure of the soluble form of the redox-regulated chloride ion channel protein CLIC4, FEBS J. 272 (2005) 4996–5007.
[33] D.R. Littler, S.J. Harrop, W.D. Fairlie, L.J. Brown, G.J. Pankhurst, S. Pankhurst, M.Z. DeMaere, T.J. Campbell, A.R. Bauskin, R. Tonini, M. Mazzanti, S.N. Breit, P.M. Curmi, The intracellular chloride ion channel protein CLIC1 undergoes a redox-controlled structural transition, J. Biol. Chem. 279 (2004) 9298–9305.
[34] L. Jiang, K. Salao, H. Li, J.M. Rybicka, R.M. Yates, X.W. Luo, X.X. Shi, T. Kuffner, V.W. Tsai, Y. Husaini, L. Wu, D.A. Brown, T. Grewal, L.J. Brown, P.M. Curmi, S.N. Breit, Intracellular chloride channel protein CLIC1 regulates macrophage function through modulation of phagosomal acidification, J. Cell Sci. 125 (2012) 5479–5488.
[35] S.C. Goodchild, C.N. Angstmann, S.N. Breit, P.M. Curmi, L.J. Brown, Transmembrane extension and oligomerization of the CLIC1 chloride intracellular channel protein upon membrane interaction, Biochemistry 50 (2011) 10887–10897.
[36] C.B. Anfinsen, Principles that govern the folding of protein chains, Science 181 (1973) 223–230.
[37] A.G. Murzin, Biochemistry. Metamorphic proteins, Science 320 (2008) 1725–1726.
[38] S.C. Goodchild, P.M.G. Curmi, L.J. Brown, Structural gymnastics of multifunctional metamorphic proteins, Biophys. Rev. 3 (2011) 143–153.
[39] P.N. Bryan, J. Orban, Proteins that switch folds, Curr. Opin. Struct. Biol. 20 (2010) 482–488.
[40] D. Legg-E'silva, I. Achilonu, S. Fanucchi, S. Stoychev, M. Fernandes, H.W. Dirr, Role of arginine 29 and glutamic acid 81 interactions in the conformational stability of human chloride intracellular channel 1, Biochemistry 51 (2012) 7854–7862.
[41] I. Achilonu, S. Fanucchi, M. Cross, M. Fernandes, H.W. Dirr, Role of individual histidines in the pH-dependent global stability of human chloride intracellular channel 1, Biochemistry 51 (2012) 995–1004.
[42] S.H. Stoychev, C. Nathaniel, S. Fanucchi, M. Brock, S. Li, K. Asmus, V.L. Woods Jr., H.W. Dirr, Structural dynamics of soluble chloride intracellular channel protein CLIC1 examined by amide hydrogen-deuterium exchange mass spectrometry, Biochemistry 48 (2009) 8413–8421.
[43] S. Fanucchi, R.J. Adamson, H.W. Dirr, Formation of an unfolding intermediate state of soluble chloride intracellular channel protein CLIC1 at acidic pH, Biochemistry 47 (2008) 11674–11681.
[44] E. Gouaux, Channel-forming toxins: tales of transformation, Curr. Opin. Struct. Biol. 7 (1997) 566–573.

[45] S.L. Schendel, M. Montal, J.C. Reed, Bcl-2 family proteins as ion-channels, Cell Death Differ. 5 (1998) 372–380.
[46] H. Singh, M.A. Cousin, R.H. Ashley, Functional reconstitution of mammalian 'chloride intracellular channels' CLIC1, CLIC4 and CLIC5 reveals differential regulation by cytoskeletal actin, FEBS J. 274 (2007) 6306–6316.
[47] K. Warton, R. Tonini, W.D. Fairlie, J.M. Matthews, S.M. Valenzuela, M.R. Qiu, W.M. Wu, S. Pankhurst, A.R. Bauskin, S.J. Harrop, T.J. Campbell, P.M. Curmi, S.N. Breit, M. Mazzanti, Recombinant CLIC1 (NCC27) assembles in lipid bilayers via a pH-dependent two-state process to form chloride ion channels with identical characteristics to those observed in Chinese hamster ovary cells expressing CLIC1, J. Biol. Chem. 277 (2002) 26003–26011.
[48] B.M. Tulk, S. Kapadia, J.C. Edwards, CLIC1 inserts from the aqueous phase into phospholipid membranes where it functions as an anion channel, Am. J. Physiol. Cell Physiol. 282 (2002) C1103–C1112.
[49] B.M. Tulk, P.H. Schlesinger, S.A. Kapadia, J.C. Edwards, CLIC-1 functions as a chloride channel when expressed and purified from bacteria, J. Biol. Chem. 275 (2000) 26986–26993.
[50] S.M. Valenzuela, H. Alkhamici, L.J. Brown, O.C. Almond, S.C. Goodchild, S. Carne, P.M.G. Curmi, S.A. Holt, B.A. Cornell, Regulation of the membrane insertion and conductance activity of the metamorphic chloride intracellular channel protein CLIC1 by cholesterol, PLoS One 8 (2013) e56948.
[51] R.K. Tweten, Cholesterol-dependent cytolysins, a family of versatile pore-forming toxins, Infect. Immun. 73 (2005) 6199–6209.
[52] A.F. Dulhunty, P. Pouliquin, M. Coggan, P.W. Gage, P.G. Board, A recently identified member of the glutathione transferase structural family modifies cardiac RyR2 substate activity, coupled gating and activation by Ca2+ and ATP, Biochem. J. 390 (2005) 333–343.
[53] P.G. Board, M. Coggan, S. Watson, P.W. Gage, A.F. Dulhunty, CLIC-2 modulates cardiac ryanodine receptor Ca^{2+} release channels, Int. J. Biochem. Cell Biol. 36 (2004) 1599–1612.
[54] X. Meng, G. Wang, C. Viero, Q. Wang, W. Mi, X.D. Su, T. Wagenknecht, A.J. Williams, Z. Liu, C.C. Yin, CLIC2-RyR1 interaction and structural characterization by cryo-electron microscopy, J. Mol. Biol. 387 (2009) 320–334.
[55] R. Tonini, A. Ferroni, S.M. Valenzuela, K. Warton, T.J. Campbell, S.N. Breit, M. Mazzanti, Functional characterization of the NCC27 nuclear protein in stable transfected CHO-K1 cells, FASEB J. 14 (2000) 1171–1178.
[56] W. Suginta, N. Karoulias, A. Aitken, R.H. Ashley, Chloride intracellular channel protein CLIC4 (p64H1) binds directly to brain dynamin I in a complex containing actin, tubulin and 14-3-3 isoforms, Biochem. J. 359 (2001) 55–64.
[57] K. Golovnina, A. Blinov, E.M. Akhmametyeva, L.V. Omelyanchuk, L.S. Chang, Evolution and origin of merlin, the product of the Neurofibromatosis type 2 (NF2) tumor-suppressor gene, BMC Evol. Biol. 5 (2005) 69.
[58] A.I. McClatchey, R.G. Fehon, Merlin and the ERM proteins–regulators of receptor distribution and signaling at the cell cortex, Trends Cell Biol. 19 (2009) 198–206.
[59] W.T. Lankes, H. Furthmayr, Moesin: a member of the protein 4.1-talin-ezrin family of proteins, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 8297–8301.
[60] E. Gould, L.L. Butcher, Developing cholinergic basal forebrain neurons are sensitive to thyroid hormone, J. Neurosci. 9 (1989) 3347–3358.
[61] N. Funayama, A. Nagafuchi, N. Sato, S. Tsukita, Radixin is a novel member of the band 4.1 family, J. Cell Biol. 115 (1991) 1039–1048.
[62] A.H. Chishti, A.C. Kim, S.M. Marfatia, M. Lutchman, M. Hanspal, H. Jindal, S.C. Liu, P.S. Low, G.A. Rouleau, N. Mohandas, J.A. Chasis, J.G. Conboy, P. Gascard, Y. Takakuwa, S.C. Huang, E.J. Benz Jr., A. Bretscher, R.G. Fehon, J.F. Gusella, V. Ramesh, F. Solomon, V.T. Marchesi, S. Tsukita, K.B. Hoover, et al., The FERM domain: a unique module involved in the linkage of cytoplasmic proteins to the membrane, Trends Biochem. Sci. 23 (1998) 281–282.
[63] A. Bretscher, K. Edwards, R.G. Fehon, ERM proteins and merlin: integrators at the cell cortex, Nat. Rev. Mol. Cell Biol. 3 (2002) 586–599.
[64] Q. Li, M.R. Nance, R. Kulikauskas, K. Nyberg, R. Fehon, P.A. Karplus, A. Bretscher, J.J. Tesmer, Self-masking in an intact ERM-merlin protein: an active role for the central alpha-helical domain, J. Mol. Biol. 365 (2007) 1446–1459.
[65] S.D. Edwards, N.H. Keep, The 2.7 A crystal structure of the activated FERM domain of moesin: an analysis of structural changes on activation, Biochemistry 40 (2001) 7061–7068.
[66] K. Hamada, T. Shimizu, T. Matsui, S. Tsukita, T. Hakoshima, Structural basis of the membrane-targeting and unmasking mechanisms of the radixin FERM domain, EMBO J. 19 (2000) 4449–4462.
[67] W.J. Smith, N. Nassar, A. Bretscher, R.A. Cerione, P.A. Karplus, Structure of the active N-terminal domain of ezrin. Conformational and mobility changes identify keystone interactions, J. Biol. Chem. 278 (2003) 4949–4956.
[68] M.A. Pearson, D. Reczek, A. Bretscher, P.A. Karplus, Structure of the ERM protein moesin reveals the FERM domain fold masked by an extended actin binding tail domain, Cell 101 (2000) 259–270.
[69] R. Gary, A. Bretscher, Ezrin self-association involves binding of an N-terminal domain to a normally masked C-terminal domain that includes the F-actin binding site, Mol. Biol. Cell 6 (1995) 1061–1075.
[70] D. Reczek, M. Berryman, A. Bretscher, Identification of EBP50: a PDZ-containing phosphoprotein that associates with members of the ezrin–radixin–moesin family, J. Cell Biol. 139 (1997) 169–179.
[71] V. Niggli, C. Andreoli, C. Roy, P. Mangeat, Identification of a phosphatidylinositol-4,5-bisphosphate-binding domain in the N-terminal region of ezrin, FEBS Lett. 376 (1995) 172–176.
[72] C. Barret, C. Roy, P. Montcourrier, P. Mangeat, V. Niggli, Mutagenesis of the phosphatidylinositol 4,5-bisphosphate (PIP(2)) binding site in the NH(2)-terminal domain of ezrin correlates with its altered cellular distribution, J. Cell Biol. 151 (2000) 1067–1080.
[73] F. Nakamura, L. Huang, K. Pestonjamasp, E.J. Luna, H. Furthmayr, Regulation of F-actin binding to platelet moesin in vitro by both phosphorylation of threonine 558 and polyphosphatidylinositides, Mol. Biol. Cell 10 (1999) 2669–2685.
[74] S. Yonemura, T. Matsui, S. Tsukita, Rho-dependent and -independent activation mechanisms of ezrin/radixin/moesin proteins: an essential role for polyphosphoinositides in vivo, J. Cell Sci. 115 (2002) 2569–2580.
[75] B.T. Fievet, A. Gautreau, C. Roy, L. Del Maestro, P. Mangeat, D. Louvard, M. Arpin, Phosphoinositide binding and phosphorylation act sequentially in the activation mechanism of ezrin, J. Cell Biol. 164 (2004) 653–659.
[76] F. Nakamura, M.R. Amieva, H. Furthmayr, Phosphorylation of threonine 558 in the carboxyl-terminal actin-binding domain of moesin by thrombin activation of human platelets, J. Biol. Chem. 270 (1995) 31377–31385.
[77] T. Matsui, M. Maeda, Y. Doi, S. Yonemura, M. Amano, K. Kaibuchi, S. Tsukita, Rho-kinase phosphorylates COOH-terminal threonines of ezrin/radixin/moesin (ERM) proteins and regulates their head-to-tail association, J. Cell Biol. 140 (1998) 647–657.
[78] P.C. Simons, S.F. Pietromonaco, D. Reczek, A. Bretscher, L. Elias, C-terminal threonine phosphorylation activates ERM proteins to link the cell's cortical lipid bilayer to the cytoskeleton, Biochem. Biophys. Res. Commun. 253 (1998) 561–565.
[79] S.F. Pietromonaco, P.C. Simons, A. Altman, L. Elias, Protein kinase C-theta phosphorylation of moesin in the actin-binding sequence, J. Biol. Chem. 273 (1998) 7594–7603.
[80] T. Ng, M. Parsons, W.E. Hughes, J. Monypenny, D. Zicha, A. Gautreau, M. Arpin, S. Gschmeissner, P.J. Verveer, P.I. Bastiaens, P.J. Parker, Ezrin is a downstream effector of trafficking PKC-integrin complexes involved in the control of cell motility, EMBO J. 20 (2001) 2723–2741.
[81] O. Turunen, T. Wahlstrom, A. Vaheri, Ezrin has a COOH-terminal actin-binding site that is conserved in the ezrin protein family, J. Cell Biol. 126 (1994) 1445–1453.
[82] Y. Takai, K. Kitano, S. Terawaki, R. Maesaki, T. Hakoshima, Structural basis of the cytoplasmic tail of adhesion molecule CD43 and its binding to ERM proteins, J. Mol. Biol. 381 (2008) 634–644.
[83] T. Mori, K. Kitano, S. Terawaki, R. Maesaki, Y. Fukami, T. Hakoshima, Structural basis for CD44 recognition by ERM proteins, J. Biol. Chem. 283 (2008) 29602–29612.
[84] K. Hamada, T. Shimizu, S. Yonemura, S. Tsukita, T. Hakoshima, Structural basis of adhesion-molecule recognition by ERM proteins revealed by the crystal structure of the radixin–ICAM-2 complex, EMBO J. 22 (2003) 502–514.
[85] Y. Takai, K. Kitano, S. Terawaki, R. Maesaki, T. Hakoshima, Structural basis of PSGL-1 binding to ERM proteins, Genes Cells 12 (2007) 1329–1338.
[86] S. Terawaki, K. Kitano, T. Hakoshima, Structural basis for type II membrane protein binding by ERM proteins revealed by the radixin-neutral endopeptidase 24.11 (NEP) complex, J. Biol. Chem. 282 (2007) 19854–19862.
[87] S. Terawaki, R. Maesaki, T. Hakoshima, Structural basis for NHERF recognition by ERM proteins, Structure 14 (2006) 777–789.
[88] C.M. Finnerty, D. Chambers, J. Ingraffea, H.R. Faber, P.A. Karplus, A. Bretscher, The EBP50-moesin interaction involves a binding site regulated by direct masking on the FERM domain, J. Cell Sci. 117 (2004) 1547–1552.
[89] A. Bretscher, D. Chambers, R. Nguyen, D. Reczek, ERM-Merlin and EBP50 protein families in plasma membrane organization and function, Annu. Rev. Cell Dev. Biol. 16 (2000) 113–143.
[90] A.S. Fanning, J.M. Anderson, Protein modules as organizers of membrane structure, Curr. Opin. Cell Biol. 11 (1999) 432–439.
[91] A. Claperon, M. Mergey, L. Fouassier, Roles of the scaffolding proteins NHERF in liver biology, Clin. Res. Hepatol. Gastroenterol. 35 (2011) 176–181.
[92] D.P. LaLonde, D. Garbett, A. Bretscher, A regulated complex of the scaffolding proteins PDZK1 and EBP50 with ezrin contribute to microvillar organization, Mol. Biol. Cell 21 (2010) 1519–1529.
[93] S. Marion, E. Hoffmann, D. Holzer, C. Le Clainche, M. Martin, M. Sachse, I. Ganeva, P. Mangeat, G. Griffiths, Ezrin promotes actin assembly at the phagosome membrane and regulates phago-lysosomal fusion, Traffic 12 (2011) 421–437.
[94] L.P. Erwig, K.A. McPhilips, M.W. Wynes, A. Ivetic, A.J. Ridley, P.M. Henson, Differential regulation of phagosome maturation in macrophages and dendritic cells mediated by Rho GTPases and ezrin–radixin–moesin (ERM) proteins, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 12825–12830.
[95] J.M. Kinchen, K.S. Ravichandran, Phagosome maturation: going through the acid test, Nat. Rev. Mol. Cell Biol. 9 (2008) 781–795.
[96] R.G. Fehon, A.I. McClatchey, A. Bretscher, Organizing the cell cortex: the role of ERM proteins, Nat. Rev. Mol. Cell Biol. 11 (2010) 276–287.
[97] K. Wennerberg, K.L. Rossman, C.J. Der, The Ras superfamily at a glance, J. Cell Sci. 118 (2005) 843–846.
[98] I.R. Vetter, A. Wittinghofer, The guanine nucleotide-binding switch in three dimensions, Science 294 (2001) 1299–1304.
[99] W.D. Heo, T. Inoue, W.S. Park, M.L. Kim, B.O. Park, T.J. Wandless, T. Meyer, PI(3,4,5)P3 and PI(4,5)P2 lipids target proteins with polybasic clusters to the plasma membrane, Science 314 (2006) 1458–1461.
[100] O. Visvikis, M.P. Maddugoda, E. Lemichez, Direct modifications of Rho proteins: deconstructing GTPase regulation, Biol. Cell 102 (2010) 377–389.
[101] A.J. Ridley, Rho GTPases and actin dynamics in membrane protrusions and vesicle trafficking, Trends Cell Biol. 16 (2006) 522–529.
[102] S.J. Heasman, A.J. Ridley, Mammalian Rho GTPases: new insights into their functions from in vivo studies, Nat. Rev. Mol. Cell Biol. 9 (2008) 690–701.
[103] M.R. Qiu, L. Jiang, K.I. Matthaei, S.M. Schoenwaelder, T. Kuffner, P. Mangin, J.E. Joseph, J. Low, D. Connor, S.M. Valenzuela, P.M. Curmi, L.J. Brown, M. Mahaut-Smith, S.P. Jackson, S.N. Breit, Generation and characterization of mice with null mutation of the chloride intracellular channel 1 gene, Genesis 48 (2010) 127–136.
[104] S. Louvet-Vallee, ERM proteins: from cellular architecture to cell signaling, Biol. Cell 92 (2000) 305–316.

[105] O. Speck, S.C. Hughes, N.K. Noren, R.M. Kulikauskas, R.G. Fehon, Moesin functions antagonistically to the Rho pathway to maintain epithelial integrity, Nature 421 (2003) 83–87.
[106] A.W. Segal, K.P. Shatwell, The NADPH oxidase of phagocytic leukocytes, Ann. N. Y. Acad. Sci. 832 (1997) 215–222.
[107] C.D. Ellson, S. Gobert-Gosse, K.E. Anderson, K. Davidson, H. Erdjument-Bromage, P. Tempst, J.W. Thuring, M.A. Cooper, Z.Y. Lim, A.B. Holmes, P.R. Gaffney, J. Coadwell, E.R. Chilvers, P.T. Hawkins, L.R. Stephens, PtdIns(3)P regulates the neutrophil oxidase complex by binding to the PX domain of p40(phox), Nat. Cell Biol. 3 (2001) 679–682.
[108] F. Kanai, H. Liu, S.J. Field, H. Akbary, T. Matsuo, G.E. Brown, L.C. Cantley, M.B. Yaffe, The PX domains of p47phox and p40phox bind to lipid products of PI(3)K, Nat. Cell Biol. 3 (2001) 675–678.
[109] K. Lapouge, S.J. Smith, P.A. Walker, S.J. Gamblin, S.J. Smerdon, K. Rittinger, Structure of the TPR domain of p67phox in complex with Rac.GTP, Mol. Cell 6 (2000) 899–907.
[110] M. Parri, P. Chiarugi, Rac and Rho GTPases in cancer cell motility control, Cell Commun. Signal. 8 (2010) 23.
[111] I. van den Bout, N. Divecha, PIP5K-driven PtdIns(4,5)P2 synthesis: regulation and cellular functions, J. Cell Sci. 122 (2009) 3837–3850.
[112] V.D. Rao, S. Misra, I.V. Boronenkov, R.A. Anderson, J.H. Hurley, Structure of type IIbeta phosphatidylinositol phosphate kinase: a protein kinase fold flattened for interfacial phosphorylation, Cell 94 (1998) 829–839.
[113] N. Divecha, Lipid kinases: charging PtdIns(4,5)P2 synthesis, Curr. Biol. 20 (2010) R154–R157.
[114] R.A. Lacalle, R.M. Peregil, J.P. Albar, E. Merino, A.C. Martinez, I. Merida, S. Manes, Type I phosphatidylinositol 4-phosphate 5-kinase controls neutrophil polarity and directional movement, J. Cell Biol. 179 (2007) 1539–1553.
[115] A. Bretscher, Purification of an 80,000-Dalton protein that is a component of the isolated microvillus cytoskeleton, and its localization in nonmuscle cells, J. Cell Biol. 97 (1983) 425–432.
[116] V.L. Bonilha, Focus on molecules: ezrin, Exp. Eye Res. 84 (2007) 613–614.
[117] I. Saotome, M. Curto, A.I. McClatchey, Ezrin is essential for epithelial organization and villus morphogenesis in the developing intestine, Dev. Cell 6 (2004) 855–864.
[118] J.B. Casaletto, I. Saotome, M. Curto, A.I. McClatchey, Ezrin-mediated apical integrity is required for intestinal homeostasis, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 11924–11929.
[119] A. Tamura, S. Kikuchi, M. Hata, T. Katsuno, T. Matsui, H. Hayashi, Y. Suzuki, T. Noda, S. Tsukita, Achlorhydria by ezrin knockdown: defects in the formation/expansion of apical canaliculi in gastric parietal cells, J. Cell Biol. 169 (2005) 21–28.
[120] M. Berryman, J. Bruno, J. Price, J.C. Edwards, CLIC-5A functions as a chloride channel in vitro and associates with the cortical actin cytoskeleton in vitro and in vivo, J. Biol. Chem. 279 (2004) 34794–34801.
[121] L.H. Gagnon, C.M. Longo-Guess, M. Berryman, J.B. Shin, K.W. Saylor, H. Yu, P.G. Gillespie, K.R. Johnson, The chloride intracellular channel protein CLIC5 is expressed at high levels in hair cell stereocilia and is essential for normal inner ear function, J. Neurosci. 26 (2006) 10188–10198.
[122] S.Y. Khan, Z.M. Ahmed, M.I. Shabbir, S. Kitajiri, S. Kalsoom, S. Tasneem, S. Shayiq, A. Ramesh, S. Srisailpathy, S.N. Khan, R.J. Smith, S. Riazuddin, T.B. Friedman, Mutations of the RDX gene cause nonsyndromic hearing loss at the DFNB24 locus, Hum. Mutat. 28 (2007) 417–423.
[123] S. Kitajiri, K. Fukumoto, M. Hata, H. Sasaki, T. Katsuno, T. Nakagawa, J. Ito, S. Tsukita, Radixin deficiency causes deafness associated with progressive degeneration of cochlear stereocilia, J. Cell Biol. 166 (2004) 559–570.
[124] F. Pataky, R. Pironkova, A.J. Hudspeth, Radixin is a constituent of stereocilia in hair cells, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 2601–2606.
[125] G.I. Welsh, M.A. Saleem, The podocyte cytoskeleton—key to a functioning glomerulus in health and disease, Nat. Rev. Nephrol. 8 (2012) 14–21.
[126] J. Patrakka, K. Tryggvason, Molecular make-up of the glomerular filtration barrier, Biochem. Biophys. Res. Commun. 396 (2010) 164–169.
[127] N. Sachs, A. Sonnenberg, Cell-matrix adhesion of podocytes in physiology and disease, Nat. Rev. Nephrol. 9 (2013) 200–210.
[128] S. Schmieder, M. Nagai, R.A. Orlando, T. Takeda, M.G. Farquhar, Podocalyxin activates RhoA and induces actin reorganization through NHERF1 and ezrin in MDCK cells, J. Am. Soc. Nephrol. 15 (2004) 2289–2298.
[129] B. Wegner, A. Al-Momany, S.C. Kulak, K. Kozlowski, M. Obeidat, N. Jahroudi, J. Paes, M. Berryman, B.J. Ballermann, CLIC5A, a component of the ezrin-podocalyxin complex in glomeruli, is a determinant of podocyte integrity, Am. J. Physiol. Renal Physiol. 298 (2010) F1492–F1503.
[130] B.A. Pierchala, M.R. Munoz, C.C. Tsui, Proteomic analysis of the slit diaphragm complex: CLIC5 is a protein critical for podocyte morphology and function, Kidney Int. 78 (2010) 868–882.
[131] J. Nystrom, W. Fierlbeck, A. Granqvist, S.C. Kulak, B.J. Ballermann, A human glomerular SAGE transcriptome database, BMC Nephrol. 10 (2009) 13.
[132] R.A. Orlando, T. Takeda, B. Zak, S. Schmieder, V.M. Benoit, T. McQuistan, H. Furthmayr, M.G. Farquhar, The glomerular epithelial cell anti-adhesin podocalyxin associates with the actin cytoskeleton through interactions with ezrin, J. Am. Soc. Nephrol. 12 (2001) 1589–1598.
[133] T. Takeda, T. McQuistan, R.A. Orlando, M.G. Farquhar, Loss of glomerular foot processes is associated with uncoupling of podocalyxin from the actin cytoskeleton, J. Clin. Invest. 108 (2001) 289–301.
[134] J.Z. Chuang, S.Y. Chou, C.H. Sung, Chloride intracellular channel 4 is critical for the epithelial morphogenesis of RPE cells and retinal attachment, Mol. Biol. Cell 21 (2010) 3017–3028.
[135] V.L. Bonilha, S.C. Finnemann, E. Rodriguez-Boulan, Ezrin promotes morphogenesis of apical microvilli and basal infoldings in retinal pigment epithelium, J. Cell Biol. 147 (1999) 1533–1548.
[136] V.L. Bonilha, M.E. Rayborn, I. Saotome, A.I. McClatchey, J.G. Hollyfield, Microvilli defects in retinas of ezrin knockout mice, Exp. Eye Res. 82 (2006) 720–729.
[137] J.J. Tung, I.W. Tattersall, J. Kitajewski, Tips, stalks, tubes: notch-mediated cell fate determination and mechanisms of tubulogenesis during angiogenesis, Cold Spring Harb. Perspect. Med. 2 (2012) a006601.
[138] K. Xu, O. Cleaver, Tubulogenesis during blood vessel formation, Semin. Cell Dev. Biol. 22 (2011) 993–1004.
[139] D.M. Bryant, A. Datta, A.E. Rodriguez-Fraticelli, J. Peranen, F. Martin-Belmonte, K.E. Mostov, A molecular network for de novo generation of the apical surface and lumen, Nat. Cell Biol. 12 (2010) 1035–1045.
[140] M. Buechner, D.H. Hall, H. Bhatt, E.M. Hedgecock, Cystic canal mutants in *Caenorhabditis elegans* are defective in the apical membrane domain of the renal (excretory) cell, Dev. Biol. 214 (1999) 227–241.
[141] B.C. Mattingly, M. Buechner, The FGD homologue EXC-5 regulates apical trafficking in *C. elegans* tubules, Dev. Biol. 359 (2011) 59–72.
[142] N. Suzuki, M. Buechner, K. Nishiwaki, D.H. Hall, H. Nakanishi, Y. Takai, N. Hisamoto, K. Matsumoto, A putative GDP–GTP exchange factor is required for development of the excretory cell in *Caenorhabditis elegans*, EMBO Rep. 2 (2001) 530–535.
[143] J. Gao, L. Estrada, S. Cho, R.E. Ellis, J.L. Gorski, The *Caenorhabditis elegans* homolog of FGD1, the human Cdc42 GEF gene responsible for faciogenital dysplasia, is critical for excretory cell morphogenesis, Hum. Mol. Genet. 10 (2001) 3049–3062.
[144] M. Fujita, D. Hawkinson, K.V. King, D.H. Hall, H. Sakamoto, M. Buechner, The role of the ELAV homologue EXC-7 in the development of the *Caenorhabditis elegans* excretory canals, Dev. Biol. 256 (2003) 290–301.
[145] P.M. Loria, A. Duke, J.B. Rand, O. Hobert, Two neuronal, nuclear-localized RNA binding proteins involved in synaptic transmission, Curr. Biol. 13 (2003) 1317–1323.
[146] X. Tong, M. Buechner, CRIP homologues maintain apical cytoskeleton to regulate tubule size in *C. elegans*, Dev. Biol. 317 (2008) 225–233.
[147] V. Gobel, P.L. Barrett, D.H. Hall, J.T. Fleming, Lumen morphogenesis in *C. elegans* requires the membrane-cytoskeleton linker erm-1, Dev. Cell 6 (2004) 865–873.
[148] D. Van Furden, K. Johnson, C. Segbert, O. Bossinger, The *C. elegans* ezrin–radixin–moesin protein ERM-1 is necessary for apical junction remodelling and tubulogenesis in the intestine, Dev. Biol. 272 (2004) 262–276.
[149] L.A. Khan, H. Zhang, N. Abraham, L. Sun, J.T. Fleming, M. Buechner, D.H. Hall, V. Gobel, Intracellular lumen extension requires ERM-1-dependent apical membrane expansion and AQP-8-mediated flux, Nat. Cell Biol. 15 (2012) 143–156.
[150] M. Kamei, W.B. Saunders, K.J. Bayless, L. Dye, G.E. Davis, B.M. Weinstein, Endothelial tubes assemble from intracellular vacuoles in vivo, Nature 442 (2006) 453–456.
[151] B. Lubarsky, M.A. Krasnow, Tube morphogenesis: making and shaping biological tubes, Cell 112 (2003) 19–28.
[152] T. Stauber, T.J. Jentsch, Chloride in vesicular trafficking and function, Annu. Rev. Physiol. 75 (2013) 453–477.
[153] B. Ulmasov, J. Bruno, N. Gordon, M.E. Hartnett, J.C. Edwards, Chloride intracellular channel protein-4 functions in angiogenesis by supporting acidification of vacuoles along the intracellular tubulogenic pathway, Am. J. Pathol. 174 (2009) 1084–1096.
[154] D. Chalothorn, H. Zhang, J.E. Smith, J.C. Edwards, J.E. Faber, Chloride intracellular channel-4 is a determinant of native collateral formation in skeletal muscle and brain, Circ. Res. 105 (2009) 89–98.
[155] G. Novarino, C. Fabrizi, R. Tonini, M.A. Denti, F. Malchiodi-Albedi, G.M. Lauro, B. Sacchetti, S. Paradisi, A. Ferroni, P.M. Curmi, S.N. Breit, M. Mazzanti, Involvement of the intracellular ion channel CLIC1 in microglia-mediated beta-amyloid-induced neurotoxicity, J. Neurosci. 24 (2004) 5322–5330.
[156] O.V. Vieira, R.J. Botelho, S. Grinstein, Phagosome maturation: aging gracefully, Biochem. J. 366 (2002) 689–704.
[157] D.G. Russell, B.C. Vanderven, S. Glennie, H. Mwandumba, R.S. Heyderman, The macrophage marches on its phagosome: dynamic assays of phagosome function, Nat. Rev. Immunol. 9 (2009) 594–600.
[158] S. Solomon, N. Rajasekaran, E. Jeisy-Walder, S.B. Snapper, H. Illges, A crucial role for macrophages in the pathology of K/B x N serum-induced arthritis, Eur. J. Immunol. 35 (2005) 3064–3073.
[159] M.A. Dozynkiewicz, N.B. Jamieson, I. Macpherson, J. Grindlay, P.V. van den Berghe, A. von Thun, J.P. Morton, C. Gourley, P. Timpson, C. Nixon, C.J. McKay, R. Carter, D. Strachan, K. Anderson, O.J. Sansom, P.T. Caswell, J.C. Norman, Rab25 and CLIC3 collaborate to promote integrin recycling from late endosomes/lysosomes and drive cancer progression, Dev. Cell 22 (2012) 131–145.
[160] C. Tringali, B. Lupo, I. Silvestri, N. Papini, L. Anastasia, G. Tettamanti, B. Venerando, The plasma membrane sialidase NEU3 regulates the malignancy of renal carcinoma cells by controlling beta1 integrin internalization and recycling, J. Biol. Chem. 287 (2012) 42835–42845.
[161] L.M. Knowles, G. Malik, B.L. Hood, T.P. Conrads, J. Pilch, CLT1 targets angiogenic endothelium through CLIC1 and fibronectin, Angiogenesis 15 (2012) 115–129.
[162] L.M. Knowles, J. Zewe, G. Malik, A.V. Parwani, J.R. Gingrich, J. Pilch, CLT1 Targets bladder cancer through integrin alpha5beta1 and CLIC3, Mol. Cancer Res. 11 (2013) 194–203.
[163] K.H. Kim, B.K. Choi, K.M. Song, K.W. Cha, Y.H. Kim, H. Lee, I.S. Han, B.S. Kwon, CRIg signals induce anti-intracellular bacterial phagosome activity in a chloride intracellular channel 3-dependent manner, Eur. J. Immunol. 43 (2013) 667–678.
[164] S. McLaughlin, A. Aderem, The myristoyl-electrostatic switch: a modulator of reversible protein-membrane interactions, Trends Biochem. Sci. 20 (1995) 272–276.
[165] G. Di Paolo, P. De Camilli, Phosphoinositides in cell regulation and membrane dynamics, Nature 443 (2006) 651–657.
[166] R. Ghai, A. Bugarcic, H. Liu, S.J. Norwood, S. Skeldal, E.J. Coulson, S.S. Li, R.D. Teasdale, B.M. Collins, Structural basis for endosomal trafficking of diverse

transmembrane cargos by PX-FERM proteins, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) E643–E652.
[167] R. Ghai, M. Mobli, S.J. Norwood, A. Bugarcic, R.D. Teasdale, G.F. King, B.M. Collins, Phox homology band 4.1/ezrin/radixin/moesin-like proteins function as molecular scaffolds that interact with cargo receptors and Ras GTPases, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 7763–7768.
[168] R. Ghai, B.M. Collins, PX-FERM proteins: a link between endosomal trafficking and signaling? Small Gtpases 2 (2011) 259–263.
[169] X. Li, R. Zhang, K.M. Draheim, W. Liu, D.A. Calderwood, T.J. Boggon, Structural basis for small G protein effector interaction of Ras-related protein 1 (Rap1) and adaptor protein Krev interaction trapped 1 (KRIT1), J. Biol. Chem. 287 (2012) 22317–22327.
[170] D.R. Littler, L.J. Brown, S.N. Breit, A. Perrakis, P.M. Curmi, Structure of human CLIC3 at 2 A resolution, Proteins 78 (2010) 1594–1600.
[171] B. Ulmasov, J. Bruno, P.G. Woost, J.C. Edwards, Tissue and subcellular distribution of CLIC1, BMC Cell Biol. 8 (2007) 8.