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Review

Model membranes to shed light on the biochemical and physical properties of ezrin/radixin/moesin

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ABSTRACT

Ezrin, radixin and moesin (ERM) proteins are more and more recognized to play a key role in a large number of important physiological processes such as morphogenesis, cancer metastasis and virus infection. Recent reviews extensively discuss their biological functions [1–4]. In this review, we will first remind the main features of this family of proteins, which are known as linkers and regulators of plasma membrane/cytoskeleton linkage. We will then briefly review their implication in pathological processes such as cancer and viral infection. In a second part, we will focus on biochemical and biophysical approaches to study ERM interaction with lipid membranes and conformational change in well-defined environments. *In vitro* studies using biomimetic lipid membranes, especially large unilamellar vesicles (LUVs), giant unilamellar vesicles (GUVs) and supported lipid bilayers (SLBs) and recombinant proteins help to understand the molecular mechanism of conformational activation of ERM proteins. These tools are aimed to decorticate the different steps of the interaction, to simplify the experiments performed *in vivo* in much more complex biological environments.

1. Biological importance of ezrin, radixin and moesin

1.1. General overview of ERM proteins (ezrin, radixin, moesin)

ERM proteins are localized at the plasma membrane in actin-rich surface structures (Fig. 1) [5], such as microvilli, membrane ruffles, and lamellipodia in gut cells, lymphocytes, hepatocytes, spermatozoids, fibroblasts and in a variety of other cell types [5–9]. They are involved in various physiological processes including establishment of cell polarity, cell motility and cell signaling [10]. They act as linkers between the plasma membrane and the cortical actin cytoskeleton. From a structural point of view, ERMs are constituted of 3 domains: a membrane binding domain, also known as FERM (for band 4.1 protein, ezrin, radixin and moesin), and intermediary region and a actin binding domain called C-ERMAD (for C-terminal ERM-association domain) (Fig. 2A).

The FERM domain, constituted of ~300 amino acids, is characteristic of the proteins of the band 4.1 superfamily. These proteins also present other types of domains, including PDZ, tyrosine phosphatase, SH2-like, tyrosine kinase, kinase-like, myosin head, and PH domains [11]. In ERM proteins, the FERM domain is followed by a long α-helical region, which forms a coiled-coil structure, according to the only structure of full-length moesin available [12] (Fig. 2A). The C-terminal domain, which is constituted of ~80 residues, is known to contain an actin binding site [13](Fig. 2A). ERM function is regulated by head to tail interactions between the FERM domain (Fig. 2B) and the C-terminal (C-ERMAD) domain (Fig. 2C). In the folded conformation, also called closed conformation (or dormant, or inactive), the F-actin binding site is masked. In the unfolded open conformation, also called active, the N-terminus binds the plasma membrane and the C-terminus is accessible for binding actin filaments [14,15].

1.2. Specific roles vs. functional redundancy

ERM proteins play an important role in the organization of the cell cortex [2]. They interact with a large range of proteins, including other cytoplasmic or membrane associated proteins, such as ezrin binding protein 50 (EBP50), also known as Na^+/H^+ exchanger regulating factor 1 (NHERF1) [16] and Na^+/H^+ exchanger type 3 kinase A regulatory protein (E3KARP or NHERF2) [17], as well as transmembrane proteins, among which CD43, CD44 and

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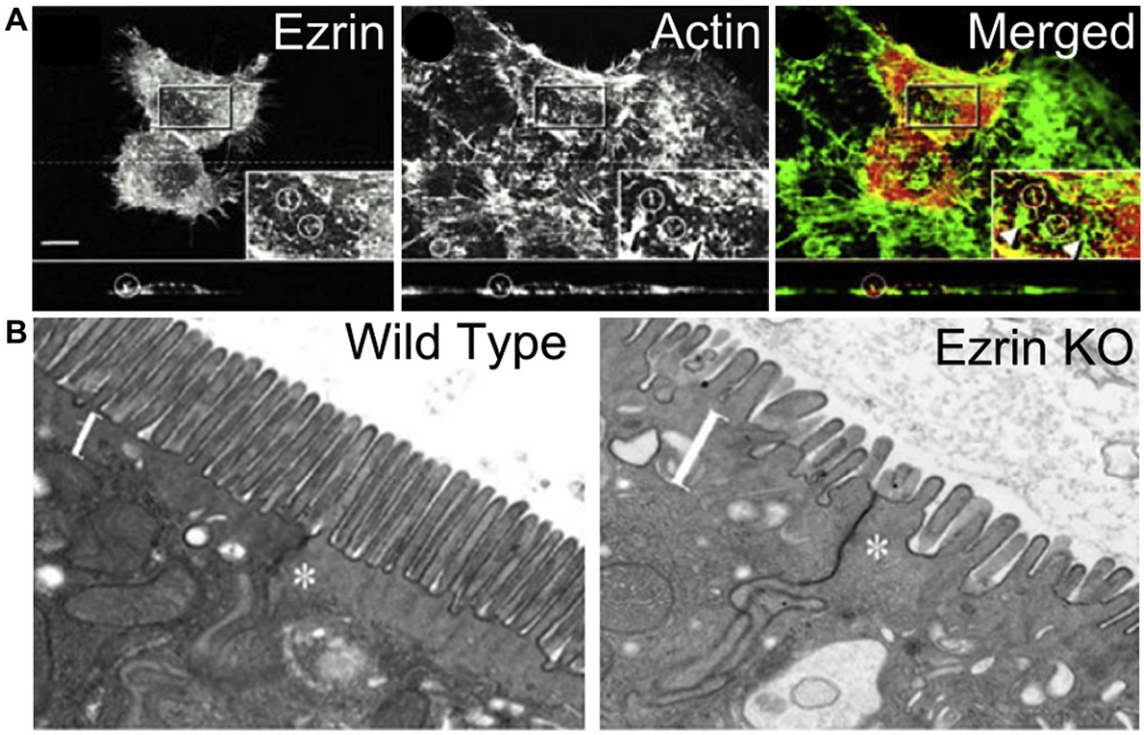

Fig. 1. (A) *In cellulo* localization of ezrin and actin in human adenocarcinoma A431 epitheloid cells. Cells were transfected with either VSV-G-tagged wild-type ezrin and treated for indirect Texas red localization of ezrin with anti-VSV antibody (left) and F-actin with FITC-coupled phalloidin. Colocalization of ezrin with actin filaments in dynamic structures appears in yellow (right) [50] (reproduced with permission from Ref. [50], copyright (2000) Rockefeller University Press 2000). (B) Transmission electron microscopy of intestinal microvilli of WT (left) and ezrin knock-out mouse (right). The wild type intestinal epithelial cells reveal packed and uniform rod-like microvilli across the apical surface whereas the microvilli of knock-out mouse cells show not well oriented, thick and non-uniform [20] (reproduced with permission from Ref. [20], copyright (2004) Elsevier).

intracellular adhesion molecule 2 (ICAM-2) [18]. All these interactions contribute to the formation of functional protein complexes to stabilize the cytoskeleton–plasma membrane linkage. Furthermore, they interact with phospholipids. In vertebrates, the three ERM proteins show tissue specific expression profiles [4]. Ezrin, first isolated in gastric parietal cells, is present mostly in epithelial cells, while moesin is mostly found in endothelial cells. Radixin is rather found in hepatocytes [2]. Bretscher *et al.* first discovered ezrin in 1983 [19] and found that it was present in very large amounts in membrane protrusions, such as intestinal microvilli. However, no clear role for its involvement in the formation of the latter appeared. To investigate more precisely the involvement of ERM in the morphogenesis of epithelial tissues, several studies using mutant mice have emerged. In mutant mice lacking ezrin, intestinal epithelium cells showed malformations: instead of having many microvilli oriented and with defined size, they were thicker, shorter and not well oriented (Fig. 1B). Consequently, the newly born mutant mice did not survive more than a few days as their intestines were unable to incorporate the essential nutrients [20]. Similarly, other reports using the same approach showed that the absence of radixin was not lethal. However, mice lacking radixin showed liver damage after 8 weeks [21]. In addition, radixin appeared to be important for the implementation of the architecture of cochlear stereocilia, but its absence was offset by other ERMs, including ezrin [22]. It seems the lack of radixin was compensated by other ERM. ERM protein functions are redundant and the redundancy was also confirmed by another study [23], which showed that mutant mice deficient in moesin exhibited no functional or structural abnormality of the tissues suggesting that the other ERM proteins take over functions of moesin. Despite their redundancy, ezrin and moesin are differentially distributed in the early steps of melanoma tumor cell invasion [24]. The different distributions of ezrin and moesin were also reported during T cell activation [25,26]. Schaffer *et al.*, reported that ezrin, but not moesin, is transiently present at the immune synapse, before movement to the distal pole complex [26]. However no specific requirements for ezrin vs. moesin in the activation process were evidenced. Ilani *et al.* showed that, upon activation of T-lymphocytes, cell shape changed and a loss of villi and actin polymerization at the interface between the lymphocyte and an antigen-presenting cell was observed. Moesin was absent from the immune synapse, while ezrin was present in this area. In this case, moesin interacted with CD43 whereas ezrin interacted with the signaling kinase ZAP-70. This study pointed out for the first time a non-redundancy in the function of ERM [25]. Recently, Haynes *et al.* [27], showed that increased moesin expression, but not ezrin or radixin, was necessary during epithelial–mesenchymal transition for efficient actin filament remodeling, and for cortical relocalization of adhesion and contractile elements. This relocalization included CD44, α-smooth muscle actinin, and phosphorylated myosin light chain [27].

1.3. ERMs in pathological processes

1.3.1. Cancer

Ezrin was first identified as a crucial molecule in the dissemination of two pediatric tumors, rhabdomyosarcoma [28] and osteosarcoma [29]. Clinical studies have shown that ezrin overexpression also correlated with adult tumor metastasis. Ezrin overexpression increased migration of metastatic melanoma [30], pancreatic cancer cells [31], or hepatocellular carcinoma [32]. Conversely, ezrin silencing or inhibition contributed to decreased cell motility [33–36]. Ezrin implication in cancer has been related to its interactions with a plethora of molecules related to metastatic functions. We will not cite all of them but a few starting with CD44,