New insights into potential functions for the protein 4.1 superfamily of proteins in kidney epithelium

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1. ABSTRACT

Members of the protein 4.1 family of adapter proteins are expressed in a broad panel of tissues including various epithelia where they likely play an important role in maintenance of cell architecture and polarity and in control of cell proliferation. We have recently characterized the structure and distribution of three members of the protein 4.1 family, 4.1B, 4.1R and 4.1N, in mouse kidney. We describe here binding partners for renal 4.1 proteins, identified through the screening of a rat kidney yeast two-hybrid system cDNA library. The identification of putative protein 4.1-based complexes enables us to envision potential functions for 4.1 proteins in kidney: organization of signaling complexes, response to osmotic stress, protein trafficking, and control of cell proliferation. We discuss the relevance of these protein 4.1-based interactions in kidney physio-pathology in the context of their previously identified functions in other cells and tissues. Specifically, we will focus on renal 4.1 protein interactions with beta amyloid precursor protein (beta-APP), 14-3-3 proteins, and the cell swelling-activated chloride channel pICln. We also discuss the functional relevance of another member of the protein 4.1 superfamily, ezrin, in kidney physio-pathology.

2. INTRODUCTION

The cytoskeleton is composed of a complex network of proteins that participates in maintenance of cell architecture and polarity through proper sorting and retention of transmembrane proteins. Thus, cytoskeletal proteins 4.1 and ezrin act as scaffold proteins by bridging an increasing list of transmembrane proteins to actin filaments and specialized adapter and signaling molecules (1-9). These cytoskeleton-based interactions are dynamic and subject to regulation depending on cell activation and cell environment. The key role of protein 4.1 in membrane architecture and function is illustrated by the observation that a dramatic decrease or loss in protein 4.1 expression results in a concomitant decrease in the level of expression and mislocalization of transmembrane and membrane-associated proteins that normally interact with protein 4.1 (10-27). Members of the protein 4.1 superfamily have been originally thought to play exclusively a structural role. There is now strong evidence for their involvement in other cellular functions, including cell polarity (3, 15, 28-31), nuclear architecture and cell division (32-45), control of cell volume in response to osmotic stress (46), and control of cell proliferation (47-53). As detailed later in this
In this review, we present evidence for potential interactions of the three major kidney protein 4.1 gene products with selected binding partners in an attempt to delineate the functions that these cytoskeletal proteins may play in kidney and in other epithelia. The potential functional relevance of protein 4.1 interactions with beta amyloid precursor protein (beta-APP), members of the 14-3-3 family of proteins, and the cell-swelling activated chloride channel pICln, will be explored. The involvement of another member of the protein 4.1 superfamily, ezrin, in kidney structure, function and pathology is also discussed in detail. Renal ankars will be evoked briefly since they are described in another chapter of this issue (see Mohler and Bennett).

3. PROTEIN 4.1 STRUCTURE

Protein 4.1 belongs to the Ezrin/Radixin/Moesin (ERM) superfamily of adapter proteins that bridge membrane proteins and actin filaments (4,54). Members of the ERM family share a homologous region, referred to as the FERM (Four.1/Ezrin/Radixin/Moesin) domain (55), suggesting that they may associate with identical or related membrane proteins. The family of 4.1 proteins is composed of prototypical red blood cell 4.1R (56-59), and three homologs, 4.1G (60, 61), 4.1N (62-65) and 4.1B (66-69).

Protein 4.1 genes differ in their expression pattern: 4.1R is predominantly expressed in hematopoietic tissues, and in regions of brain and kidney (66, 70, 71); 4.1G shows a broad distribution (60, 61, 70) but, unlike the three other 4.1 proteins, it is not expressed in kidney (71, 72); 4.1N is mostly neuron- and kidney- specific with low expression in other tissues. The four protein 4.1 genes have been mapped on both man and mouse chromosomes (66, 69). They share a similar domain organization characterized by the existence of multiple initiation sites combined with a large number of alternative exons. This results in the generation of a broad repertoire of isoforms from each protein 4.1 gene (30, 34, 58, 59, 71, 76-93).

Well-defined structural domains have been delineated within the protein 4.1 coding region (Figure 1) (94). As illustrated in Figure 2, the 30kD FERM domain, also referred to as MBD domain, mediates interaction of 4.1R and/or its homologs with numerous binding partners including various transmembrane proteins such as the anion exchanger AE1 (or band 3) (95-97), glycophorin C (25, 98-105), CD44 (23), nectin (106) and Neuroexins such as Paranodin (107-110), PDZ domain-containing proteins p55 (12, 19, 111), discs large (Dlg) protein, also referred to as synapse-associated protein 97 (SAP97) (31, 112, 113), and CASK (29, 114), cytoskeletal protein tubulin (115, 116), a swelling-activated chloride channel pICln, will be explored. The involvement of another member of the protein 4.1 superfamily, ezrin, in kidney structure, function and pathology is also discussed in detail. Renal ankars will be evoked briefly since they are described in another chapter of this issue (see Mohler and Bennett).

Figure 1. Domain organization of 4.1 proteins. Alignment of 4.1 proteins reveals the presence of three conserved domains (FERM domain, spectrin-actin binding (SAB) domain, and C-terminal domain (CTD)) highlighted in shades of grey and of three unique regions (U1, U2, U3) displayed as white boxes. Numbers in boxes refer to percent of identity of a given conserved domain of 4.1G, 4.1N and 4.1B to the corresponding 4.1R domain. The 4.1R map shows a unique region U3 that is only expressed in epithelial tissues. Note that the SAB domain is not conserved in 4.1N.

Mouse 4.1R FERM domain

MICKVSSLDDTVPVECEVEHHAKQGDLLKRVCEHLMLEL DYYFLAIWNTSKEKKEQKQVCRGVPVNFTKFINK YPPDEPAQLTEDITYYLCQLRQDIVGRPRFATLLGLS YTIQSEGLBDYDPELHGVDYYDFKLAPNQTELEFKVM FIGHKSYRMSPAPOADLEELFEKALKILSMVYDIHKAKD LECVDHGLCVSSEGLVYKDKLRINRFPWPKVLKSYKRS SFFIKIRPGEOFOFSTIGFLIPSTRAAKKLWKNVCFYEFH TFERLUMSTUTPKSFLACSGSKF

Figure 2. Mapping of the binding motifs for various binding partners in protein 4.1 FERM domain. We have highlighted known binding motifs for various binding partners in protein 4.1 FERM domain to illustrate the close proximity of these motifs and therefore the possibility of steric competition depending on cellular context. A Glu-Glu-Asp (EED) motif highlighted in red in exon 5-encoded peptide mediates interaction with anion exchanger AE1 (95). A 47 mer peptide encoded by exon 8, highlighted in purple, mediates 4.1 interaction with glycophorin C (24). Exon 9- and 11-encoded peptides highlighted in green mediate 4.1 interaction with calmodulin (46). Exon 10-encoded peptide highlighted in blue mediates 4.1 interaction with tubulin (116) and with PDZ domain-containing proteins p55 (111) and likely Dlg (SAP 97) and CASK. A large peptide encompassing exons 9-11 (underlined in the sequence) is necessary for 4.1 interaction with pICln (46). The Tyr residue outlined in exon-11 encoded peptide in 4.1R corresponds to the location of the Phe responsible for 4.1B, 4.1N and 4.1G interaction with 14-3-3 proteins (50). The loss of this Phe residue in 4.1R explains the weak affinity of 4.1R for 14-3-3 proteins compared to the three other 4.1 proteins.

review, the complex gene organization of the four members of the protein 4.1 family likely plays a major role in tissue and cellular specialization of 4.1 proteins.
interactions of ERM proteins with PIP2 play a key role in glycogen synthase (288). Phosphorylation site K/R-X-X-S/T previously described in these two residues are components of the canonical PKC whose activity is modulated by the FERM domain and the terminal domain (CTD) interacts with various proteins including receptors (13, 14, 17, 18, 20, 26, 27), tight junction proteins (135) and the nuclear mitotic apparatus-associated protein NuMA (36, 42). The four protein 4.1 genes show high homology in their FERM, SAB (except for 4.1N), and CTD domains (Figure 1), suggesting that they may share common functions.

In contrast, the unique regions U1, U2 and U3, interspersed between the conserved domains, may confer specific regulatory functions upon each 4.1 protein (Figure 1). The unique region U1 has been shown to modulate 4.1R nuclear translocation (34, 35, 134). This region also interacts with calmodulin in a calcium-dependent manner (136) and with a centrosomal-associated protein (38). The unique region U1 also harbors a phosphorylation site for the cyclin-dependent cdc2 kinase (37), the level of phosphorylation of 4.1R varying during the cell cycle. No binding partners for the U2 and U3 unique regions have been identified so far. The unique region U2 contains a key Ser residue that is the primary substrate for protein kinase C (PKC)-dependent phosphorylation of 4.1R (137). Importantly, phosphorylation of this Ser residue leads to a decrease in 4.1R interaction with transmembrane protein glycoporphin C and with spectrin and actin. Given the conservation of this Ser residue and of the surrounding amino acids in all four 4.1 proteins (Figure 1), one may anticipate that PKC-dependent phosphorylation will play a key role in regulation of 4.1G, 4.1N and 4.1B function as well. Importantly, the U2 region has been recently shown to confer upon 4.1B its anti-proliferative properties through a still unknown mechanism (51). No function has been assigned to the U3 region yet. Of particular note, inclusion or exclusion of this alternative U3 region is a hallmark of epithelial 4.1R and 4.1N, respectively (30, 71). This intriguing feature likely reflects unique properties assigned to each of these protein 4.1 gene products in epithelia, including kidney. The emerging concept is that the unique regions act as modulators of protein 4.1 interactions mediated by the conserved domains and that tissue- and cell-specific splicing patterns in those regions confer upon each protein isoform unique characteristics.

4. CYTOSKELETON AND KIDNEY ARCHITECTURE AND FUNCTION

The nephron is composed of a monolayer of highly polarized epithelial cells that are uniquely suited to perform specific transport functions. The nonrandom distribution of membrane proteins reflects the vectorial transport functions performed by these cells (41-43), and the key role played by the cytoskeleton in targeting and positioning membrane proteins in these cells (7, 138-144). Importantly, while such protein complexes exist as soluble cytoplasmic scaffolds in non-confluent cells, they are targeted to cell-cell contact regions of the membrane in confluent cells, namely the lateral domain in the kidney epithelium, under the control of cell adhesion molecules (145-150). The apical membrane of the kidney epithelium is also divided into microdomains in which proteins segregate, also through interactions with the cytoskeleton (151-158). Those general concepts are likely to be applicable to most epithelia.

4.1. Renal 4.1 proteins

We have recently established that three members of the protein 4.1 family, i.e 4.1B, 4.1R and 4.1N, are expressed in mouse kidney (71). We have extensively characterized the kidney-specific splicing events in each protein 4.1 gene and the consequences of these splicing events on the structure of kidney protein 4.1 isoforms. Thus, compared to the full length proteins displayed in Figure 1, kidney 4.1B and kidney 4.1R both lack the U1 region. In addition, all three proteins lack small regions at the boundary of the U2 region and the SAB domain. Moreover, kidney 4.1B lacks a small N-terminal peptide in the U3 region and kidney 4.1N lacks most of the U3 region. Lastly, kidney 4.1B bears a truncation of the very end of the C-terminal domain (66, 71, 88). The absence of defined domains in renal 4.1 proteins likely confers upon their structure specific features that meet functional requirements in the context of the kidney epithelium. Indeed, we and others have gathered evidence for some of these kidney-specific splicing events either impairing or facilitating interactions of protein 4.1 isoforms with specific binding partners. For example, the major isoform of kidney 4.1R lacks a small peptide encoded by exon 16. Since this peptide contains a spectrin-binding motif (93, 129, 159, 160) and a nuclear localization signal (32, 33, 131, 161), we predict that this kidney 4.1R isoform will interact weakly with spectrin and will show low level of nuclear
An intriguing feature of renal 4.1 proteins is their mutually exclusive expression along the nephron: 4.1B is primarily expressed in the proximal convoluted tubule (PCT) and the glomerulus, while 4.1R is detected in the thick ascending limb (TAL) of the loop of Henle, and 4.1N in the thin limb of the loop of Henle, the distal convoluted tubule (DCT) and all regions of the collecting duct (71, 72). At the cellular level, all 4.1 proteins are detected in the basolateral region of the kidney epithelium. Given the extensive knowledge of transport functions dedicated to each segment of the nephron, these observations strongly suggest that each 4.1 protein is likely involved in the organization of region-specific protein scaffolds and therefore likely plays unique functions in kidney.

Interestingly, 4.1R null mice display not only the expected hematopoietic phenotype (chronic hemolytic anemia, splenomegaly, spherocytosis and reticulocytosis; (162)) but also neuro-behavioral deficits, likely resulting from the lack of 4.1R expression in cerebellum, dentate gyrus and hippocampus (70), and a renal phenotype (our unpublished data). Indeed, 4.1R null mice present with a slight urinary acidification and alterations in Na/K balance upon water deprivation. More recently, we have reported that 4.1R null red blood cells display hyperactivity of the sodium-proton exchanger NHE1 (163), of the “Gardos channel” calcium-gated potassium channel (163, 164) and of a potassium-chloride co-transporter (163). Terada et al. have also suggested an interaction between 4.1B and the sodium bicarbonate co-transporter NBC1 in the PCT based on electron microscopy analysis of kidney sections showing co-localization of the two proteins (72). Taken together, these recent findings provide us with interesting leads as for identifying additional ion transporters interacting with 4.1 proteins in the kidney epithelium.

Figure 4. Distinct distribution of protein 4.1B and ezrin or NHERF-1 in mouse kidney proximal convoluted tubules. Staining of paraformaldehyde-fixed mouse kidney sections was performed as previously described (71) using a goat polyclonal anti 4.1B antibody (66) diluted at 3 micrograms/ml in conjunction with either a rabbit polyclonal anti-ezrin antibody (Upstate USA, Inc., Charlottesville, VA) or a rabbit polyclonal anti-NHERF-1 antibody kindly provided by Dr. Stephen Lambert (University of Massachusetts, Worcester, MA) both diluted at 4 micrograms/ml. Primary antibodies were detected using anti-goat IgGs coupled to fluorescein isothiocyanate and anti-rabbit IgGs coupled to Texas Red. As previously reported, protein 4.1B shows exclusive basolateral distribution in proximal convoluted tubules (71) while ezrin and NHERF-1 are both expressed at the apical pole of the tubules. The tubules lacking 4.1B expression but stained with anti ezrin and anti-NHERF-1 antibodies correspond to other regions of the nephron.

4.2. Renal ezrin

A member of the protein 4.1 superfamily, ezrin, is expressed at high levels at the apical pole of epithelia including kidney (158) (Figure 4). Up-regulation of ezrin expression is a hallmark of major kidney diseases such as polycystic kidney disease (165) and nephrogenic diabetes insipidus (166). Ezrin, in association with PDZ domain containing protein sodium-proton exchanger regulatory factor 2 (NHERF-2), bridges the cytoskeleton and the integral membrane protein podocalyxin in podocytes (167), disorganization of this link leading to a dramatic loss in glomerular foot processes (168). A NHERF-2/podocalyxin complex has also been recently characterized in the apical region of MDCK cells undergoing polarization (169). In a similar fashion, in concert with PDZ domain-containing protein NHERF-1, ezrin has been shown to regulate sodium and phosphate reabsorption and proton secretion at the apical pole of the proximal convoluted tubule through its interactions with the sodium/phosphate co-transporter Npt2 and the sodium proton exchanger NHE3, respectively (170, 171). Thus, ezrin binds to the cytoplasmic domain of NHE3 but recruits also adapter and signaling molecules, i.e. NHERF-1 and cyclic AMP-dependent protein kinase (PKA) (172-174). Recruitment of PKA in the vicinity of NHE3 represents a key event in the regulation of NHE3 exchanger activity. Alterations in ezrin expression and PKA signaling have been observed in polycystic kidney disease (165).

Co-staining of mouse kidney with an anti 4.1B antibody and either an anti-ezrin or an anti-NHERF-1 antibody illustrates the mutually exclusive expression of renal 4.1 proteins and ezrin or NHERF-1 at the basolateral and apical pole of the kidney epithelium, respectively (Figure 4). This specialized epithelial organization may play an important role in coordination of ion transport. Indeed the level of activity of NHE3 at the apical pole of the TAL epithelium has been demonstrated to depend on the level of activity of NHE1 at the basolateral pole (175-177), the integrity of the cytoskeleton being required for this functional link to operate (178). The potential
Protein 4.1 in kidney physio-pathology

Table 1. Potential binding partners for renal 4.1 proteins in kidney

<table>
<thead>
<tr>
<th>Baits</th>
<th>Preys</th>
<th>Number of clones</th>
<th>Site of interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>kidney 4.1B</td>
<td>14-3-3theta</td>
<td>13/32</td>
<td>FERM</td>
</tr>
<tr>
<td></td>
<td>14-3-3zeta</td>
<td>7/32</td>
<td>FERM</td>
</tr>
<tr>
<td></td>
<td>14-3-beta</td>
<td>7/32</td>
<td>FERM</td>
</tr>
<tr>
<td></td>
<td>pCln</td>
<td>5/32</td>
<td>FERM</td>
</tr>
<tr>
<td>kidney 4.1R</td>
<td>LRP16</td>
<td>6/16</td>
<td>CTD</td>
</tr>
<tr>
<td></td>
<td>14-3-3beta</td>
<td>2/16</td>
<td>FERM</td>
</tr>
<tr>
<td></td>
<td>14-3-theta</td>
<td>1/16</td>
<td>FERM</td>
</tr>
<tr>
<td></td>
<td>SEC14L1</td>
<td>1/16</td>
<td>not determined</td>
</tr>
<tr>
<td></td>
<td>Rab-GDalpha</td>
<td>2/16</td>
<td>not determined</td>
</tr>
<tr>
<td></td>
<td>beta-Amyloid Precursor Protein</td>
<td>1/16</td>
<td>CTD</td>
</tr>
<tr>
<td></td>
<td>Elongation Initiation Factor 1alpha</td>
<td>2/16</td>
<td>CTD</td>
</tr>
<tr>
<td></td>
<td>Tumor suppressor TMEM24</td>
<td>1/16</td>
<td>not determined</td>
</tr>
<tr>
<td>CTD 4.1R</td>
<td>alphaB-crystallin</td>
<td>4/8</td>
<td>CTD</td>
</tr>
<tr>
<td></td>
<td>LRP16</td>
<td>2/8</td>
<td>CTD</td>
</tr>
<tr>
<td></td>
<td>Elongation Initiation Factor 1alpha</td>
<td>2/8</td>
<td>CTD</td>
</tr>
<tr>
<td>kidney 4.1N</td>
<td>14-3-3beta</td>
<td>12/17</td>
<td>FERM</td>
</tr>
<tr>
<td></td>
<td>14-3-theta</td>
<td>3/17</td>
<td>FERM</td>
</tr>
<tr>
<td></td>
<td>14-3-zeta</td>
<td>2/17</td>
<td>FERM</td>
</tr>
</tbody>
</table>

The number of clones refers to the number of clones corresponding to the prey of interest relative to the total number of relevant clones pulled out with the bait. A total of $8 \times 10^6$, $33 \times 10^6$ and $27 \times 10^6$ Trp (+) Leu (+) yeast clones were obtained for kidney 4.1B, kidney 4.1R and kidney 4.1N baits, respectively. Among those, 237, 578 and 415 clones were β-galactosidase (+) Trp (+) Leu (+) His (+), respectively. We have screened so far 74, 100 and 65 clones, respectively. Prior to screening of the library, we ascertained that none of the baits was able by itself to confer upon yeast β-galactosidase activity and growth in absence of tryptophane, leucine and histidine. CTD: C-terminal domain; FERM: Four 4.1/Ezrin/Radixin/Moesin.

relevance of this intriguing distribution in control of cell proliferation will be discussed later in this chapter.

4.3. Renal ankyrins

Structure and function of renal ankyrins are discussed in another chapter of this issue. We will only emphasize here that, like renal 4.1 proteins: i) renal ankyrins are expressed as products of distinct ankyrin genes at the basolateral pole of the kidney epithelium; ii) these ankyrin gene products actually correspond to various isoforms generated by tissue-specific pre-mRNA splicing events; iii) renal ankyrins show mutually exclusive expression along the nephron; and iv) renal ankyrins mediate proper anchorage of key ion transporters and cell adhesion molecules in the basolateral plasma membrane of the kidney epithelium.

5. POTENTIAL FUNCTIONS FOR RENAL 4.1 PROTEINS

As a first step to decipher the roles of 4.1 proteins in kidney structure and function, we have begun to identify potential binding partners for kidney 4.1B, kidney 4.1R and kidney 4.1N, through the screening of a rat kidney yeast two-hybrid system cDNA library with full length renal 4.1 proteins (71) and 4.1R CTD baits.

5.1. Methodology

A rat kidney yeast two-hybrid system cDNA library, cloned into the GAL4 activation domain vector pGAD3S-2X (179, 180), was screened for binding partners using baits corresponding to cDNAs encoding full length coding regions of the major kidney-specific isoforms of mouse 4.1R, 4.1B and 4.1B (71), or the C-terminal domain of mouse 4.1R. Baits were cloned into the LexA DNA binding domain vector pLEX12 (179, 180) and used for yeast transformation. Yeast transformed with each pLEX12 bait cDNA, was selected in absence of Tryptophane, then transformed with the pGAD3S-2X rat kidney cDNA library conferring upon yeast growth in absence of Leucine. Yeast was grown on triple selection plates lacking Tryptophane, Leucine and Histidine in order to select clones in which the bait of interest and putative preys interacted with each other. Such protein-protein interactions restore a fully active GAL4 transcription factor and drive the expression of histidine selection and btea-galactosidase reporter genes. Selected clones were further screened for prey interaction with kidney 4.1 protein baits based on standard X-galactose filter assay (179, 180). beta-galactosidase positive clones were then characterized by DNA sequencing using a forward pGAD3S-2X vector specific primer. Interactions were re-confirmed in yeast co-transformed with cDNAs coding for the bait and the prey of interest. Further mapping of the regions in 4.1 proteins responsible for the identified interactions was determined after probing preys with various truncated variants of protein 4.1 FERM and CTD domains. Only relevant clones (i.e. with prey coding sequences in frame) are presented below.

5.2. Identification of binding partners for renal 4.1 proteins

The results of our screen to date are summarized in Table 1. The predominant binding partners for kidney 4.1B (27/32 clones) and exclusive binding partners for kidney 4.1N (17/17 clones) correspond to three members of the 14-3-3 family of proteins: 14-3-3theta, 14-3-3zeta, and 14-3-3beta. In contrast, these proteins represent a minor category of binding partners for kidney 4.1R (3/16 clones).
Protein 4.1 in kidney physio-pathology

Another binding partner for kidney 4.1B (5/32 clones) is pICln, a cell swelling-activated chloride channel previously reported to interact with 4.1R (46). Additional potential binding partners for kidney 4.1R include: a putative phosphatase suspected to promote cell proliferation, LRP16 (6/16 clones) (181, 182); two proteins involved in trafficking, SEC14L1 (1/16 clones), a mammalian homolog of yeast phosphatidylinositol/ phosphatidylinositol transfer protein SEC14 (183) and the Rab GDP-dissociation inhibitor (GDI) Rab-GDIalpha (2/16 clones), a regulatory protein for small GTP-binding Rab proteins that regulates vesicle-mediated cellular transport through control of Rab GDP/GTP exchange reaction and translocation of Rab proteins between the cytosol and cell membranes (184-190). Of particular note, a small GTPase of the Rab family has been recently shown to regulate postsynaptic terminal trafficking of AMPA receptors (191), a class of receptors that has been reported to interact with 4.1N and PDZ domain-containing protein SAP 97 (20). Another 4.1R binding partner identified is the beta-Amyloid Precursor Protein (beta-APP) (1/16 clones), a key protein in Alzheimer's disease (AD) pathogenesis (192). 4.1R may also potentially bind to a component of the protein translation machinery involved in cytoskeleton reorganization and cell transformation, i.e. elongation initiation factor 1alpha (2/16 clones) (193, 194). Lastly, we identified the putative tumor suppressor TMEM24 (195) (1/16 clones), a gene present in the 11q23.3 locus and frequently deleted in neuroblastomas, as a potential binding partner for 4.1R.

While it will be important to confirm the various protein-protein interactions identified by other methodological approaches, some of them have been previously documented in the literature. As such, we will focus our discussion on the following three classes of binding partners: beta-APP, 14-3-3 proteins, and the cell swelling-activated chloride channel pICln.

5.3. beta-Amyloid Precursor Protein: a key element in progression of neuropathies and potentially epitheliopathies

Progressive cerebral deposition of the amyloid beta-peptide (A-beta peptide) is an early and invariant feature of Alzheimer's disease (196). This peptide originates from proteolytic cleavage of the very C-terminal region of the beta-amyloid precursor protein (beta-APP), a widely expressed membrane-spanning glycoprotein. Normal secretion of beta-APP involves proteolytic cleavage, releasing the soluble extramembranous portion and retaining a 10kD C-terminal fragment in the membrane. Another proteolytic processing pathway involves reinternalization of mature beta-APP from the cell surface and its targeting to endosomes/lysosomes. Subsequent proteolytic cleavage of mature beta-APP in the late Golgi and/or endosomal compartment leads to secretion of small peptides, including the 4kD A-beta peptide, into the extracellular milieu. Mutations in beta-APP have been shown to promote its proteolysis, resulting in the hypersecretion of A-beta peptide by cells, a pre-symptomatic event in AD pathogenesis.

Accumulation of the A-beta peptide occurs not only in the brain but also in the abluminal basement membrane of brain microvessels (197) and, potentially, in epithelia, such as kidney. Indeed, the Madin Darby canine kidney (MDCK) cell line has been used to study beta-APP sorting and processing (196-199). A Tyr residue (Tyra53) and the immediately adjacent residues (654-664) in the C-terminal region of beta-APP have been shown to dictate proper basolateral sorting of the protein and secretion of the A-beta peptide in MDCK cells. Importantly, basolateral secretion of A-beta peptide in MDCK cells is disturbed by alterations of intracellular pH and by the introduction of a mutation in beta-APP associated with familial Alzheimer's disease. However, the impact of the accumulation of the A-beta peptide in the basement membrane of epithelia, including the kidney epithelium, of AD patients has not been fully evaluated.

Interestingly, 4.1R has been previously reported to co-localize with neurofibrillary tangles in brain lesions of patients presenting with AD (200). It must be emphasized that, at the time of this discovery, none of the three protein 4.1 homologs were characterized and that the antibody used in this study may have possibly cross-reacted with other 4.1 homologs, since all four 4.1 proteins are expressed in brain (66, 70). Moreover, neurofibrillary tangles have been reported to be enriched in 14-3-3zeta (201), one of the 14-3-3 isoform identified as a binding partner for kidney 4.1B and 4.1N in our screening (Table 1). The observation that 4.1R null mice display some neuro-behavioral deficits (70) highlights the importance of 4.1 proteins in brain physio-pathology.

Our yeast two-hybrid screening has identified the very last nine C-terminal amino acids of beta-APP (Y687KFFEQMQN) as the binding motif for 4.1R. Although this motif is located downstream of the region required for proper sorting of beta-APP and secretion of the A-beta peptide, one may speculate that 4.1R, and/or its homologs, could facilitate beta-APP anchorage in the basolateral membrane of various epithelia, including kidney. One may also speculate a role for 4.1R in basolateral secretion of the A-beta peptide since there is now clear evidence for the presence of a cytoskeletal network, including protein 4.1 homologs, on the cytoplasmic face of the Golgi apparatus and intracellular vesicles (4, 60-62, 202, 203).

Lastly, it is worth highlighting that beta-APP metabolism is stimulated by metabotropic glutamate receptors (204) and that its processing is regulated by these receptors in hippocampal neurons (205), a region of the brain where several protein 4.1 homologs are detected (66). Strikingly, metabotropic glutamate receptors have been recently reported to interact with 4.1G (17) and may potentially interact with other protein 4.1 homologs as well. Further studies are needed to determine whether protein 4.1 plays an important role in beta-APP metabolism and cell sorting in brain as well as in epithelia, and whether alterations in protein 4.1 expression either facilitate or impair the progression of beta-APP-related pathologies such as AD.
5.4. Cell signaling: importance of 14-3-3 proteins

As detailed earlier, several protein-protein interactions involving protein 4.1 FERM domain have now been extensively documented (Figure 2). The recent characterization of an interaction between 4.1B FERM domain and members of the family of 14-3-3 proteins adds to the diversity of protein complexes organized around the FERM domain (117). Interestingly, 4.1B FERM domain appears to interact weakly with 14-3-3 proteins compared to 4.1B and 4.1G FERM domains. A Phe residue (Glu-Gln-Phe 359-Glu) is necessary for 4.1B FERM domain interaction with 14-3-3 epsilon, 14-3-3 eta and 14-3-3 gamma (50). Alignment of the four 4.1 protein FERM domains revealed that this Phe residue is conserved in 4.1B, 4.1G and 4.1N but not in 4.1R where it is replaced by a Tyr residue (Figure 2). Importantly, a Phe 359->Tyr mutation in 4.1B FERM domain impairs its interaction with 14-3-3 proteins, providing a mechanistic explanation for the weak interaction of 4.1R with 14-3-3 proteins.

Our yeast two-hybrid system screening showed that 14-3-3 proteins are major binding partners for 4.1N. The conservation of the key Phe 359 residue in 4.1N FERM domain supports further the likeliness of interactions between 4.1N and 14-3-3 proteins in vivo. Interestingly, the repartition of 14-3-3 clones differs between kidney 4.1N and kidney 4.1B. Indeed, 14-3-3 beta appears as the major binding partner for kidney 4.1N, while 14-3-3 theta is the predominant binding partner for kidney 4.1B (Table 1). It is likely that variations in amino acids surrounding the canonical Phe 359 residue account for preferential interactions between specific 14-3-3 and protein 4.1 isoforms. It should be noted that the subset of 14-3-3 protein isoforms (14-3-3-beta, theta and zeta) that we identified to interact with 4.1 proteins are different from those identified by Yu et al. (117), i.e. 14-3-3 epsilon, eta and gamma. This may reflect tissue-specific expression of subsets of 14-3-3 isoforms. The observations emphasized above, i.e. distinct proportion of 14-3-3 clones among binding partners for each renal 4.1 protein and distinct repartition of the 14-3-3 isoform species interacting with kidney 4.1N and 4.1B, illustrate the functional specialization of 4.1 proteins despite the high level of conservation of key functional domains.

For example, protein 4.1-dependent recruitment of 14-3-3 proteins could participate in regulation of activities of transmembrane proteins such as ion channels and calcium pumps (227). A recent study has unveiled a novel role for 14-3-3 proteins in mediating downregulation of Na,K-ATPase activity upon stimulation of kidney cells by dopamine, 14-3-3 proteins recruiting PI3-kinase to the site of Na,K-ATPase endocytosis (228). The location of the 14-3-3 binding motif in 4.1 FERM domain suggests potential binding competition of 14-3-3 proteins with neighboring binding partners of 4.1 FERM domain, such as calmodulin or PDZ-domain-containing proteins (12, 19, 31, 111), depending on the cell context (Figure 2). Some of these dynamic interactions could be either favored or inhibited upon cell activation. Indeed, cell activation leads to an increase in cytosolic calcium concentration and in changes in protein phosphorylation levels, both events modulating protein 4.1 interactions with its binding partners (1, 24, 37, 111, 137, 229-232). Finally, interactions of selected 4.1 proteins with 14-3-3 isoforms could participate in the organization of other protein complexes located in cellular compartments other than the plasma membrane (233). One of those components could be the centrosome where protein 4.1 and 14-3-3 homologs have been detected (38, 39, 42, 45, 233, 234).

5.5. Regulation of cell volume in response to osmotic stress: role of the cell swelling-activated chloride channel pICln

5.5.1. Mechanisms of regulation of cell volume

Regulation of cell volume is a critical function in kidney where the epithelium environment is characterized by very different ionic compositions, depending on the segment of the nephron (235). Defective cell volume regulation leads to major nephropathies (236, 237). Changes in cell volume are accompanied by a reorganization of the actin cytoskeleton which is associated with membrane ion transporters, the cytoskeleton acting as both a structural network and a sensor for cell volume changes. Cells maintain a constant volume despite variations in concentrations of salts in their surroundings through either Regulatory Volume Increase (RVI), activated when cells shrink, or Regulatory Volume Decrease (RVD), activated when cells swell.

Two types of transporters mediate RVI, the Na,K,2Cl co-transporter BSC1 and the sodium proton exchangers NHE1, NHE2 and NHE4 (238-240). The reorganization of the actin cytoskeleton plays an important role in NaCl entry into the cell through activation of these membrane channels (241-243). In an hypotonic environment, cells undergo RVD as a result of a loss of K and Cl, mediated by activation of a K,Cl co-transporter (244). This ion efflux drives an outward movement of water molecules and subsequently a decrease in cell volume. An outward current of chloride involving also other osmolytes, known as I_{swell}, is involved in RVD as well (245). Several cell swelling-activated proteins have been proposed to account for the I_{swell} current: chloride channels CIC-2 and CIC-3, P-glycoprotein, and importantly in the context of this review, the chloride channel pICln.
5.5.2. Properties of the cell swelling-activated chloride channel pICln

5.5.2.1. pICln: a versatile protein

pICln is an ubiquitous protein essential for cell viability (246). Its over-expression results in an increase in outward Cl conductance, while its down-regulation leads to a delay and decrease in RVD (247-250). pICln therefore plays a critical role in RVD, a function that is altered in major nephropathies. pICln has been identified in reticulocytes and in young red blood cells where it accumulates in the plasma membrane through an interaction with the actin cytoskeleton (251). In platelets, pICln associates with the alpha2-beta3 integrin and regulates platelet function (252). In kidney, pICln is detected predominantly in the cytosol of all regions of the nephron (253). In addition, it is detected at the apical pole of the epithelium in the cortex, and at both basolateral and apical poles in the medulla (253). While it is primarily present in the cytosol of resting cells, it is targeted to and inserted into plasma membrane in response to cell swelling and to diuretics (253, 254). Once inserted into the membrane, pICln forms a channel-like structure enabling efflux of Cl and other osmolytes, and therefore RVD.

The mechanism by which pICln is targeted to the membrane is still unknown (253). However, the carboxyl terminus of pICln has been shown to bind to 4.1R FERM domain, suggesting a potential involvement of 4.1R in regulation of cell volume through proper membrane anchorage of pICln (46). Moreover, pICln binds to actin (251), to a non-muscle isoform of myosin light chain (255), and to a mammalian homolog of yeast Skb1 (256). Importantly, Skb1 has been shown to control cell volume in yeast (257), through cytoskeletal rearrangement mediated by its interaction with the p21^{Cdc42/Rac} activated protein kinase Shk1 (258). In summary, pICln is involved in distinct cellular processes including regulation of cell volume, cell morphology, cytoskeleton architecture, cell cycle and RNA processing. Thus defining of the functional relevance of pICln/4.1 interaction in various cellular structures is an important goal of future studies.

5.5.2.2. Characterization of pICln/4.1 interaction

Protein pICln has been previously reported to interact in vitro and in vivo with 4.1R FERM domain (46). However, the recent characterization of pICln distribution in rat kidney suggests that pICln may interact with other 4.1 proteins since it is expressed in regions of the nephron where 4.1 proteins other than 4.1R are present (71).

Interestingly, we identified pICln as a binding partner for kidney 4.1B (Table 1). As shown in Figure 5, additional binding assays performed in yeast revealed that full length kidney 4.1R (panel A1), kidney 4.1B (panel A4), kidney 4.1N (panel B1), their respective FERM domains (panels A2, A5 and B2), as well as 4.1G FERM domain (panel A6) all interact strongly with pICln. pICln interaction with each renal 4.1 protein may be therefore functionally relevant. Moreover, serial truncations of 4.1N FERM domain (panels B3-B6) enabled us to narrow down the minimal region in 4.1N FERM domain required for interaction with pICln to a peptide encompassing amino acids 162-280. A previous study reported that a slightly larger region of 4.1R FERM domain encompassing amino acids 136-283 was necessary for interaction with pICln (46). This region corresponds to the C-terminal half of the FERM domain. As proposed earlier for 14-3-3 proteins, the regulation of pICln binding to protein 4.1 FERM domain
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may also depend on the dynamic regulation of interactions of neighboring binding partners, in particular PDZ domain containing proteins and calmodulin (Figure 2).

Validation of the novel protein 4.1-mediated protein-protein interactions described above in \textit{in vivo} will rely primarily on: i) investigation of the phenotypes resulting from protein 4.1 gene silencing in relevant kidney epithelial cells, cells down-regulated for protein 4.1 expression being expected to display mis-localization of binding partners, impact on 14-3-3-mediated cell signaling, metabolism of beta-APP and cell response to osmotic stress; ii) confirmation of the phenotypes unveiled through this cell based-strategy in mouse knock out models for each protein 4.1 gene as they will become available to us. Deciphering such phenotypes will be greatly facilitated by the rapid progress in microarray-based renal systems biology (259). Such an approach will enable us defining signatures as for alterations in transcriptional activity and in transduction pathways in cell lines subjected to protein 4.1 gene silencing and in protein 4.1 null mouse kidneys.

6. PROTEIN 4.1 AND EZRIN: KEY PLAYERS IN CONTROL OF CELL PROLIFERATION

As described earlier in this review, major kidney pathologies have been linked to ezrin over-expression. To date, none have been related to alterations in levels of protein 4.1 expression despite the presence of several members of the protein 4.1 family in the nephron. We anticipate that protein 4.1-related kidney diseases, resulting from protein 4.1-dependent defects in membrane anchorage and/or protein processing as suggested here for AD, will be characterized in the near future. Such kidney diseases will very likely include proliferative diseases. Indeed, anti-proliferative properties have been extensively documented for 4.1B (51, 53), and to a lesser extent for 4.1R (48, 49) and 4.1N (260-262). Loss of heterozygosity (LOH) of 4.1B and 4.1R genes has been implicated in progression of subsets of ependymomas and meningiomas (48, 49). Some lung and breast carcinomas are also characterized by LOH and/or hypermethylation of the 4.1B gene (263). Protein 4.1B could exert its anti-proliferative effect through inhibition of protein arginine N-methyltransferase 3 activity (52). Importantly, 4.1B over-expression in proliferative neuronal cells blocks their growth (264). In a similar fashion, we have recently established that over-expression of a kidney-specific isoform of 4.1N, in a kidney epithelial cell line that normally expresses this isoform, leads to cell arrest (260). Two reports have described a concomitant loss of 4.1B and E-cadherin in cell-cell contact regions upon transition from adenoma to carcinoma in pancreas and colon epithelia (53, 73). We therefore predict that the loss of expression of renal 4.1 proteins could lead to proliferative diseases affecting primarily regions of the nephron where each 4.1 protein is solely expressed, i.e. PCT upon loss of 4.1B expression, TAL upon loss of 4.1R expression and DCT and collecting duct upon loss of 4.1N expression.

Several proteins that have been previously described as binding partners for 4.1 proteins, and other potential binding partners that co-distribute with 4.1 proteins in the lateral domain of epithelial cells, likely participate in the formation of elaborate protein complexes responsible for cell-cell adhesion and contact inhibition. They include i) cell adhesion molecules CD44, TSLC1 and the E-cadherin/beta-catenin/alpha-catenin complex (23, 147, 265, 266), ii) the sodium-proton exchanger NHE1 (267); iii) PDZ-domain containing proteins dig (SAP 97) and CASK (29, 31); and iv) cytoskeletal proteins such as spectrin elf (268). Alterations in activities of the small GTP-binding proteins Rac and RhoA have been proposed to initiate the disorganization of actin filaments observed in proliferative epithelia (269). Alterations in the actin cytoskeleton would in turn lead to the loss of adhesive protein complexes responsible for epithelial cell interactions through their lateral domains. It remains to be established whether the depolymerization of actin filaments is sufficient to induce disorganization of cell adhesion protein complexes or whether the loss of protein 4.1 in plasma membrane represents a key event in triggering such a disorganization.

The concept that the Four.1/Ezrin/Radixin/Moesin (FERM) domain, a highly conserved peptide among members of the protein 4.1 superfamily, mediates regulation of cell proliferation (270), has been recently challenged. Indeed, a recent study has established that the unique region U2 of 4.1B is actually responsible for inhibition of cell growth, the FERM domain-dependent anchorage of the U2 region to the plasma membrane representing a necessary event for inhibition of cell proliferation (51). Other mechanisms of control of cell proliferation mediated by protein 4.1 have been proposed. Thus, 4.1N nuclear translocation, occurring upon rat pheochromocytoma PC12 cell differentiation induced by nerve growth factor (261), has been shown to block cell proliferation through interaction of 4.1N CTD with the phosphatidylinositol-3 (PI-3) kinase enhancer PIKE, this interaction resulting in an inhibition of PIKE (262). A similar inhibition of PI-3 kinase through interaction of the tumor suppressor merlin with PIKE has been recently reported (271). It is likely that the FERM domain, the unique region U2 and the CTD domain of 4.1 proteins fulfill complementary functions in control of cell proliferation. In contrast with the loss of protein 4.1 expression observed in proliferative tissues, ezrin has been shown to be rather over-expressed upon tumorigenesis (272, 273). Furthermore, ezrin has been proposed to facilitate cell invasiveness and consequently to play a crucial role in metastasis (274-276). Ezrin metastatic property could result from its ability to activate the PI-3 kinase/AKT survival pathway (277), Rac1- and Ras-dependent pathways (278, 279) and the TOR/S6 kinase/4EB1 pathway (280).

An intriguing observation is the dramatic change in activities of signaling molecules such as RhoA and Rac, and of adhesion molecules such as CD44 or NHE1, depending on the cell context, i.e. arrested vs. proliferative cells. This so-called cell context would actually depend in a large part on the cross-talk between the extracellular matrix and the cell (281), this cross-talk having a big impact on the characteristics of plasma membrane protein complexes
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interacting with the cytoskeleton. Indeed, the proliferative cellular microenvironment mimics a serum deprivation that confers increased cell motility and invasion in breast cancer cells by activating NHE1 (282). NHE1 activation and the subsequent invasive phenotype of metastatic human breast cells appears to be coordinated by a sequential RhoA/p160ROCK/p38MAPK signaling pathway gated by direct PKA-dependent phosphorylation and inhibition of RhoA. The authors suggest that serum deprivation dynamically remodels the cell and compartmentalizes the signal module described above in leading-edge pseudopodia suggesting a topographic relationship between key signaling protein complexes and an invasion-specific cell structure.

The disorganization of physiological protein 4.1-based cytoskeletal complexes and the resulting formation of pathological ezrin-based cytoskeletal complexes (283) could play a key role in cell proliferation and invasiveness, by altering irreversibly key signaling cues such as the PKA-gated RhoA/p160ROCK/p38MAPK signaling pathway. In support of this hypothesis, a member of the protein 4.1 superfamily, moesin, has been recently shown to participate in epithelial organization and regulation of cell proliferation through inhibition of Rho activity, thus promoting actin polymerization (284). Other pathways could be involved as well. Thus, the Drosophila protein 4.1 homolog, coracle, has been initially discovered as a dominant suppressor of the Drosophila EGF receptor homolog (28). The concept that cytoskeletal proteins can have a direct impact on cell signaling has been reinforced by an elegant study establishing a crucial role for spectrin elf, an homolog of red cell beta-spectrin, in regulation of the Transforming Growth Factor-beta signaling pathway, through proper sorting of two key transducers of this pathway, Smad3 and Smad4 (268). These findings challenge the paradigm that members of the protein 4.1 superfamily act primarily as structural proteins by providing increasing evidence for a key role of these proteins in cell signaling through proper positioning of regulatory molecules.

The role of 4.1 proteins as tumor suppressors may also result from the ability of these proteins to maintain proper cell volume and intracellular homeostasis through the regulation of ion transporters they interact with, i.e. NHE1 and pICln. Indeed, there is now clear evidence that proliferative cells display an increase in cell volume and intracellular alkalinization (285, 286). The loss in protein 4.1 expression observed upon epithelial tumor progression, could lead to mislocalization and/or deregulation of key ion transporters that normally interact with protein 4.1. Thus, NHE1 could become hyperactive in absence of protein 4.1, resulting in an increase in NaCl reabsorption and in proton secretion, i.e. intracellular alkalinization. Such an hypothesis is supported by a recent observation highlighting NHE1 hyperactivity in 4.1R null red blood cells (163). Although the functional relevance of an interaction between another member of the protein 4.1 superfamily, ezrin, and NHE1 has been extensively documented in fibroblasts, the occurrence of this interaction in normal epithelia is unlikely given the mutually exclusive expression of NHE1 and ezrin, at the basolateral and apical poles, respectively (165, 267). Based on protein 4.1 and NHE1 co-distribution in the lateral domain of epithelia, on the presence of a potential juxta-membrane protein 4.1 binding motif (VKKKQ) in NHE1 cytoplasmic domain and on functional alterations of NHE1 in 4.1R null red blood cells (163), we are currently investigating whether 4.1R and its homologs are indeed relevant binding partners for NHE1 in kidney epithelium. We speculate that, upon loss of cell polarity and loss of protein 4.1 expression, an interaction between NHE1 and ezrin would be favored and could potentially participate in tumor progression (Figure 6).

Dissecting the protein complexes organized around ezrin and transmembrane proteins NHE1 and CD44 would likely lead to an understanding of how ezrin promotes cell motility (287). A major challenge will be to understand the mechanisms governing distinct cellular distribution and opposite effects on cell proliferation for protein 4.1 and ezrin, despite some structural and functional homology of these two cytoskeletal proteins.

7. CONCLUSION

Much remains to be done to define the actual involvement of cytoskeletal protein 4.1- and ezrin-based networks in kidney function and the mechanisms by which they fulfill these functions in various parts of the nephron. In particular, we need to further our understanding of the relationships between cytoskeleton-dependent positioning of key transmembrane and adapter proteins and cell signaling wiring and of the consequences of alterations of the cytoskeleton on those processes. The generation of mouse knock out models for each of the four protein 4.1 genes will be instrumental in evaluating the impact of a selective loss of protein 4.1 expression in vivo and in assessing the occurrence of compensatory mechanisms through up-regulation of non targeted 4.1 proteins and/or other members of the protein 4.1 superfamily, such as the ERM proteins.

A major challenge of future investigation will be to decipher the mechanisms responsible for the highly selective expression of spliceforms arising from closely related cytoskeletal protein genes in specialized regions of a given tissue and to evaluate the physiological implications of such an elaborate sorting. Such mechanisms likely involve a well orchestrated network of tissue- and cell-specific transcriptional and signaling events. Their characterization will represent a breakthrough in the cytoskeleton field and will without a doubt further our understanding of the functional links between tissue architecture and physiology. As emphasized by Mohler and Bennett in the chapter related to ankyrins, it will be crucial in that respect to choose appropriate cell and animal models to decipher this fascinating machinery.

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Figure 6. Reorganization of plasma membrane protein complexes upon epithelial cell proliferation. In a normal polarized epithelial cell, CD44, and potentially NHE1, are anchored in the lateral domain through their interaction with 4.1 proteins and the actin cytoskeleton. PDZ domain-containing proteins, such as dlg and/or CASK, would be also recruited in such complexes through interactions with 4.1 proteins (left panel). Ezrin interacts with apical transmembrane proteins such as NHE3 and recruits PDZ domain-containing proteins such as NHERF-1. Ezrin is therefore unable to interact with CD44 and NHE1 due to the mutually exclusive expression of those proteins in a different cell compartment, i.e apical pole vs. basolateral pole. Upon tumorigenesis, protein 4.1 is lost leading to disorganization of protein complexes present in lateral cell-cell contact regions and loss of cell polarity. In contrast, ezrin and its binding partner NHERF-1 become overexpressed and can now associate with CD44 and NHE1 since the localization of these proteins is no longer mutually exclusive within the plasma membrane (right panel). The formation of these novel interactions, in concert with alterations in cell microenvironment, would result in alterations of CD44 and NHE1 properties (as illustrated by changes in color patterns of those proteins between the left and the right panels) and in the formation of protein complexes that would promote cell proliferation and cell invasiveness. We speculate that a similar mechanism would occur in most proliferative epithelia, and in particular in kidney.

9. REFERENCES

Protein 4.1 in kidney physio-pathology

Protein 4.1 in kidney physio-pathology

and functional characterization of protein 4.1B, a novel member of the protein 4.1 family with high level, focal expression in brain, J Biol Chem 275, 3247-3255 (2000)
68. Yamakawa, H. & Ohara, O.: Comparison of mRNA and protein levels of four members of the protein 4.1 family: the type II brain 4.1/4.1B/KIAA0987 is the most predominant member of the protein 4.1 family in rat brain. Gene 248, 137-145 (2000)
Protein 4.1 in kidney physio-pathology


123. Han BG, W Nunomura, Y Takakuwa, N Mohandas & BK Jap: Protein 4.1R core domain structure and insights
154. Morales FC, Y Takahashi, EL Kreimann & MM Georgescu: Ezrin-radixin-moesin (ERM)-binding
Protein 4.1 in kidney physio-pathology


182. Zhao YL, WD Han, Q Li, YM Mu, XC Lu, L Yu., HJ Song, X Li, JM Lu & CY Pan: Mechanism of
transcriptional regulation of LRP16 gene expression by 17-


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