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Review

Adducin: structure, function and regulation

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Abstract. Adducin is a ubiquitously expressed membrane-skeletal protein localized at spectrin-actin junctions that binds calmodulin and is an in vivo substrate for protein kinase C (PKC) and Rho-associated kinase. Adducin is a tetramer comprised of either α/β or α/γ heterodimers. Adducin subunits are related in sequence and all contain an N-terminal globular head domain, a neck domain and a C-terminal protease-sensitive tail domain. The tail domains of all adducin subunits end with a highly conserved 22-residue myristoylated alanine-rich C kinase substrate (MARCKS)-related domain that has homology to MARCKS protein. Adducin caps the fast-growing ends of actin filaments and also preferentially recruits spectrin to the ends of filaments. Both the neck and the MARCKS-related domains are required for these activities. The neck domain self-associates to form oligomers. The MARCKS-related domain binds calmodulin and contains the major phosphorylation site for PKC. Calmodulin, gelsolin and phosphorylation by the kinase inhibit in vitro activities of adducin involving actin and spectrin. Recent observations suggest a role for adducin in cell motility, and as a target for regulation by Rho-dependent and Ca^{2+}-dependent pathways. Prominent physiological sites of regulation of adducin include dendritic spines of hippocampal neurons, platelets and growth cones of axons.

Key words. Membrane skeleton; adducin; spectrin; actin.

Introduction

The spectrin-based membrane skeleton was discovered and extensively studied in erythrocytes [1, 2]. When human erythrocytes are extracted with nonionic detergent, a dense meshwork of protein that retains the original cell shape remains after solubilization of lipids [3]. A highly organized structure of the membrane skeleton is observed by electron microscopy [4–6]. The protein organization of the membrane skeleton has been resolved in terms of the interactions among the major proteins (fig. 1). Spectrin, an extended rod-shape protein, is the principal component of the membrane skeleton. Five or six spectrin molecules are linked to short actin filaments to form a sheet of polygonal arrangements under the cytoplasmic surface of plasma membrane. The accessory proteins of the spectrin-actin network exert functions in forming connections with the membranes, promoting spectrin-actin interactions and regulating actin filaments [2, 7].

The membrane skeleton is connected to the plasma membrane of erythrocytes mainly through two classes of protein-protein interactions. The first linkage is provided by the high affinity binding between ankyrin and the β subunit of spectrin [8, 9]. Ankyrin, a peripheral

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membrane protein, also binds to the cytoplasmic domain of an integral membrane protein, the anion ex changer band 3 [9, 10]. A second membrane-spectrin linkage is mediated by protein 4.1, which binds to N-terminus of β spectrin and also binds to the cytoplasmic domain of the integral protein glycophorin C [8, 11, 12].

The proteins at the spectrin-actin junction include adducin, tropomodulin, tropomyosin, protein 4.1 and protein 4.9 [2, 7]. These proteins play essential roles in assembly and regulation of actin filaments and spectrin-actin network. The actin protofilament is proposed to be maintained by tropomyosin, adducin and tropomodulin [7]. Tropomyosin serves as a molecular ruler which defines the length of actin protofilaments [13]. Adducin caps the fast-growing ends of actin filaments, and tropomodulin caps the slow-growing ends [14, 15]. Besides linking the spectrin skeleton to the membrane, protein 4.1 stabilizes spectrin-actin interactions [8, 16]. Protein 4.1 is distinguished from adducin by its high affinity binding to spectrin in the absence of actin, and thus does not selectively recognize spectrin-actin complexes [17].

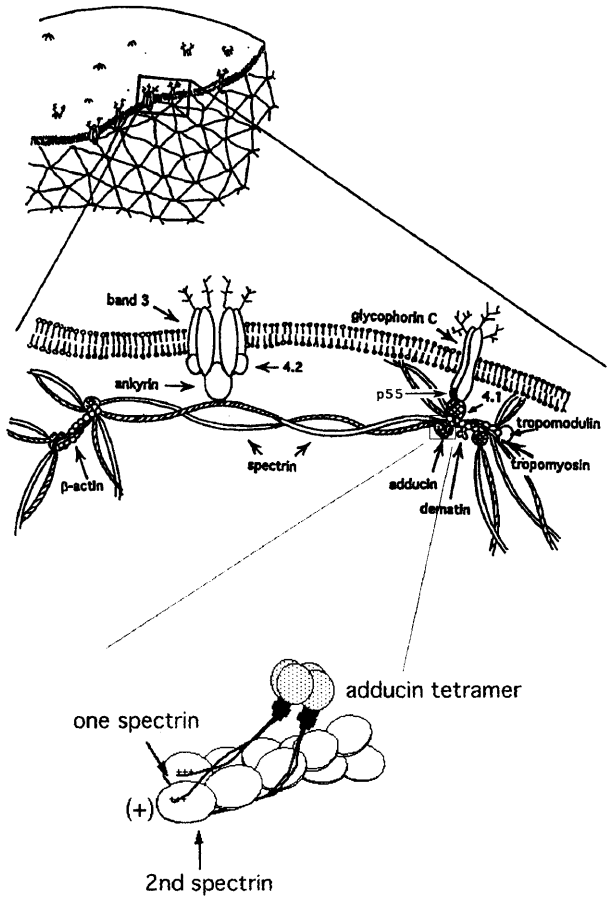

Figure 1. A schematic model of protein organization of human erythrocyte membranes and a physical model of spectrin/actin/adducin complex. The model is modified from [2].

Spectrin and its associated proteins have been found in almost all cell types of metazoan organisms [1, 2]. In cells more complex than erythrocytes, more specialized functions of membrane skeletal proteins are anticipated because of the following special features. First, major proteins show gene diversity. Spectrin, adducin, ankyrin and protein 4.1 all have families of genes and isoforms discovered in different organisms, cells and even in special cell domains. Second, the membrane skeleton in complex cells displays diversity of accessory proteins and protein-protein interactions, most of which are not observed in erythrocytes. Third, the membrane skeleton of complex cells shows diversity of localization, including specialized membrane domains and cytoplasmic organelles. The proposed functions of the spectrin-based membrane skeleton in complex cells include providing the membrane with stability and flexibility, establishing cell-cell junctions, participating in specialized membrane domains, and regulating vesicle-membrane interactions and movements of integral proteins [1, 2].

Unlike other supramolecular assembly systems such as actin, microtubules and intermediate filaments in which the backbone molecules self-assemble into higher-order structures, spectrin and actin form an irregular, tangled mass with no resemblance to the spectrin skeleton in erythrocytes when mixed in vitro [20]. In addition, the low affinity ($K_d = 6$ μM) of binding between spectrin and actin [21] suggests that the spectrin-actin network requires additional factors to assemble. Adducin selectively binds to the spectrin-actin network and recruits additional spectrins to actin filaments (see below). Thus, adducin has been proposed to function as a crucial assembly factor of spectrin-actin network in erythrocytes [19, 30].

Structure, function and regulation of adducin

Adducin genes

Adducin is encoded by three closely related genes termed α, β and γ adducin which encode polypeptides of 726, 713 and 674 amino acids, respectively [22, 23]. α and γ adducin are expressed in most tissues, whereas β adducin is most abundant in erythrocytes and brain. The primary sequences of α and β adducin display 49% identity and 66% similarity at the amino acid level. γ adducin shares 60–70% sequence similarity with either α or β adducin. All three adducin proteins contain an N-terminal globular head domain, a neck domain and a C-terminal protease-sensitive tail domain [22–25] (fig. 2). At the end of tail domain there is a 22-residue

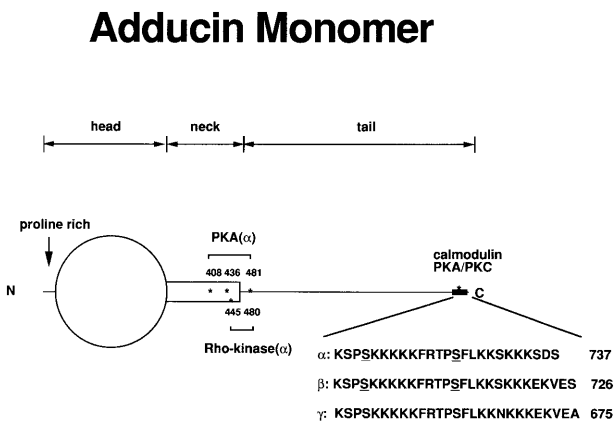

Figure 2. A model of adducin monomer based upon [22, 24]. The calmodulin-binding domain is indicated as a solid box, and the amino acid sequences of the C-terminal MARCKS-related domain of α, β and γ adducin are also presented. The major phosphorylation sites of PKA, PKC and Rho-kinase are indicated by asterisks. The phosphorylation sites of PKC identified in the MARCKS-related domain are underlined. PKA and Rho-kinase preferentially phosphorylate the Ser/Thr residues, which are indicated with their residue numbers, in and close to the neck domain of α adducin.

MARCKS-related domain that has high homology to myristoylated alanine-rich C kinase substrate (MARCKS) protein. The MARCKS-related domain has clusters of lysine residues and is highly conserved among the three adducin subunits.

α adducin has been mapped by positional cloning to human chromosome 4p16.3 [26]. The human α adducin gene contains 16 exons and spans about 85 kb of genomic DNA. Two alternatively spliced isoforms of α adducin have been identified. One α isoform results from a 93-base insertion in between codons 471 and 472, resulting in inclusion of an alternatively sliced exon (exon 15). The second alternatively spliced form introduces 3 bp within codon 621, disrupting the reading frame, and introducing a stop codon after 11 novel amino acids [26].

Human β adducin has 13 exons and is localized to chromosome 2p13–p14 [27]. Isoforms of β adducin can be divided into two groups: members of group 1 all contain the MARCKS-related domain, whereas members of group 2 lack this domain [28]. In both groups, there are three identical splices which have deletion of exons 6–9, deletion of exons 2–6 and deletion of exons 2–8.

Adducin isolated from erythrocytes is a mixture of heterodimers and heterotetramers [25]. Adducin oligomers in erythrocytes comprise α/β subunits, and in other cells include α/γ as well as α/β combinations of subunits [18, 23]. Oligomerization appears to be necessary for adducin activities, and no monomeric adducin has been observed in all types of cells tested so far [18, 29]. Oligomerization sites of adducin are located in the neck domain and probably in the head domain as well [25, 29 and also see below].

There is little information regarding functions of the head domain, even though this is the most conserved domain among adducin subunits. It is noteworthy that this domain shows sequence similarity to *Escherichia coli* fuculose-1-phosphate aldolase (AAC75842). This enzyme has a zinc binding site, and three of the histidines involved in metal coordination are conserved in the adducin head-domain sequence. The residue required for catalysis is not conserved in adducin, suggesting that adducin is not an enzyme of this type. These considerations suggest that adducin will require zinc ion for function of the head domain, and that previous in vitro studies conducted with EDTA-containing buffers may have inactivated the head domain.

In vitro activities and regulation

Adducin preferentially recruits spectrin to the fast-growing ends of actin filaments. The activities of adducin determined in in vitro assays have included recruiting spectrin to actin filaments [19, 25, 30], bundling actin filaments [31, 32] and capping the fast-growing ends of actin filaments [14]. Each of these individual adducin activities may reflect different aspects of adducin functions in living cells. It is likely that actin-capping and spectrin-recruiting activities are two aspects of one adducin/actin interaction and that spectrin is recruited by adducin to the ends and sides of actin. These hypotheses are supported by the observation that both actin-capping and spectrin-recruiting activities are inhibited by calmodulin binding [14, 19] and by protein kinase C (PKC) phosphorylation [33].

Our laboratory has recently reported that adducin promotes recruitment of spectrin to gelsolin-sensitive sites at fast-growing ends of actin filaments with a half-maximal activity of 15 nM, and to gelsolin-insensitive sites with half-maximal activity of 75 nM [29]. Adducin also exhibits a preference for actin filament ends in direct binding assays; the half-maximal concentration for binding to filament ends is 60 nM, whereas the K_d for total actin binding is 1.5 μM. The concentration of adducin of 60 nM required for half-maximal binding to filament ends is in the same range as the concentration of 150 nM required for half-maximal actin-capping activity [29]. These results are summarized in a schematic model in figure 3.

The activities of adducin imply that an important role of adducin in cells is to form a complex with the fast-growing ends of actin filaments that recruits spec-

trin and prevents addition or loss of actin subunits. Adducin is the first example of an actin-capping protein that recruits other proteins to actin filament ends, and could represent a new class of assembly factor with the function of integrating actin into other cell structures. Kuhlman and Fowler [34] have recently found that actin filament ends in intact erythrocytes are inaccessible to the actin-capping protein CapZ, which is exclusively localized in the cytosol. However, extraction of adducin from the ghost in a low ionic strength buffer lacking Mg^{2+} exposes binding sites for CapZ. These findings are consistent with capping of actin filament ends (fast-growing ends) by adducin in unlysed erythrocytes.

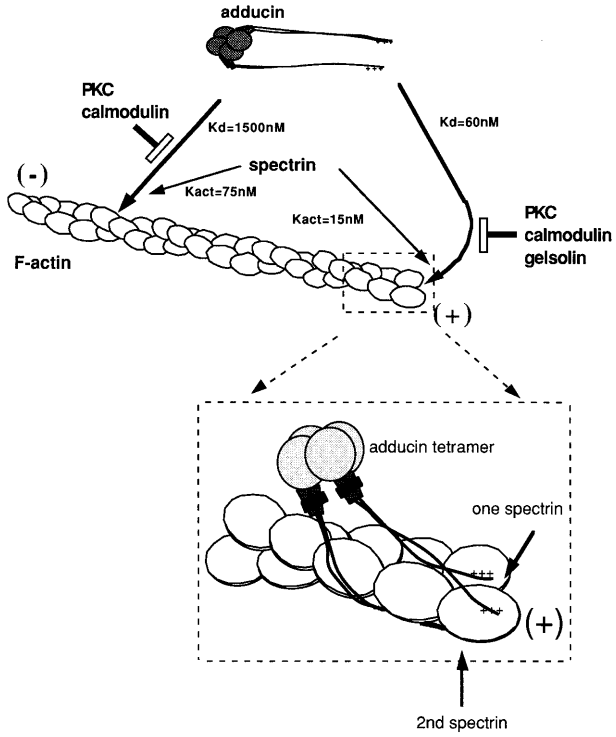

Figure 3. A schematic model of adducin association with F-actin and spectrin. Top: The model for native adducin tetramer is modified from previous studies [25]. The gelsolin-sensitive actin binding activity of adducin is high affinity (half-maximal binding at 60 nM) and low capacity (0.06 adducin/actin). The gelsolin-insensitive actin binding has low affinity ($K_d = 1.5$ μM), but a capacity as high as one adducin monomer/actin subunit, suggesting binding of adducin to the sides of actin filaments. Spectrin is recruited to the barbed end (+) of F-actin by adducin at a half-maximal activation of 15 nM adducin ($K_{act} = 15$ nM). Spectrin is also recruited to the sides of actin filaments with a $K_{act} = 75$ nM adducin. Bottom: The details of the adducin/actin complexes at the barbed end of one actin filament are illustrated. The neck domains of adducin mediate the formation of oligomers, at least homodimers. Two homodimers (α and β) further form a tetramer. The parallel orientation of adducin subunits in the oligomers is arbitrary. The MARCKS-related domain is inferred to contact actin subunits directly with 1:1 capacity. Both the neck and the MARCKS-related domain are required for the full activities of adducin.

The MARCKS-related domain is required for the activities of adducin. The neck-tail domains of β adducin (aa335–726) and native erythrocyte adducin exhibit an equivalent binding extent and binding affinity (half-maximal at 50 nM) for spectrin-recruiting activity. β 335–726 blocks 70–80% of the actin polymerization, an extent similar with that of native erythrocyte adducin. However, the estimated K_{cap} for β 335–726 is 150 nM, whereas the K_{cap} of erythrocyte adducin is about 80 nM [29]. These results demonstrate that activities of adducin with regard to spectrin recruitment and actin-capping locate in the neck-tail domains of a single subunit without apparent requirement for the head domain or heterodimers/tetramers. Similar results for α-adducin neck-tail domains are also observed.

β adducin 335–694, which lacks the C-terminal MARCKS-related domain, has almost no activity in promoting association of spectrin with actin filaments or in capping the ends of F-actin, and shows an 80% reduction in the extent of actin-binding activity [29 and fig. 4]. Therefore, the MARCKS-related domain is required for actin-binding, actin-capping and spectrin-recruiting activities of adducin. However, the MARCKS-related domain is not sufficient for these activities since β 437–726, the entire tail domain without the neck domain, exhibits minimal spectrin-recruiting, actin-capping and actin-binding activities [29 and fig. 4].

The MARCKS family of proteins has been studied extensively as in vivo substrates of PKC [35, 36]. A 25-amino acid basic domain is the site for both PKC phosphorylation and calmodulin binding of the MARCKS protein, and is similar in sequence to the MARCKS-related domain of adducin [35, 36]. The MARCKS protein binds and cross-links actin in in vitro assays, and its capacity to cross-link actin is regulated by PKC phosphorylation and calmodulin binding to the phosphorylation site domain [37]. The PKC-phosphorylation site domain of the MARCKS protein is also involved in its cell membrane binding through the interaction between a cluster of basic residues and acidic phospholipids such as phosphatidylserine (PS) [38, 39]. Phosphorylation of the serine residues in the poly-basic domain reduces its electrostatic interaction with the phospholipids, and has been proposed to provide an electrostatic switch mechanism for the reversible binding of the MARCKS protein to the cell membrane. Adducin is also a PS-binding protein [40, 41]. The MARCKS-related domain may be responsible for PS binding in a PKC-phosphorylation-dependent manner [41].

The MARCKS-related domain is a candidate to provide a direct contact to spectrin-actin due to the

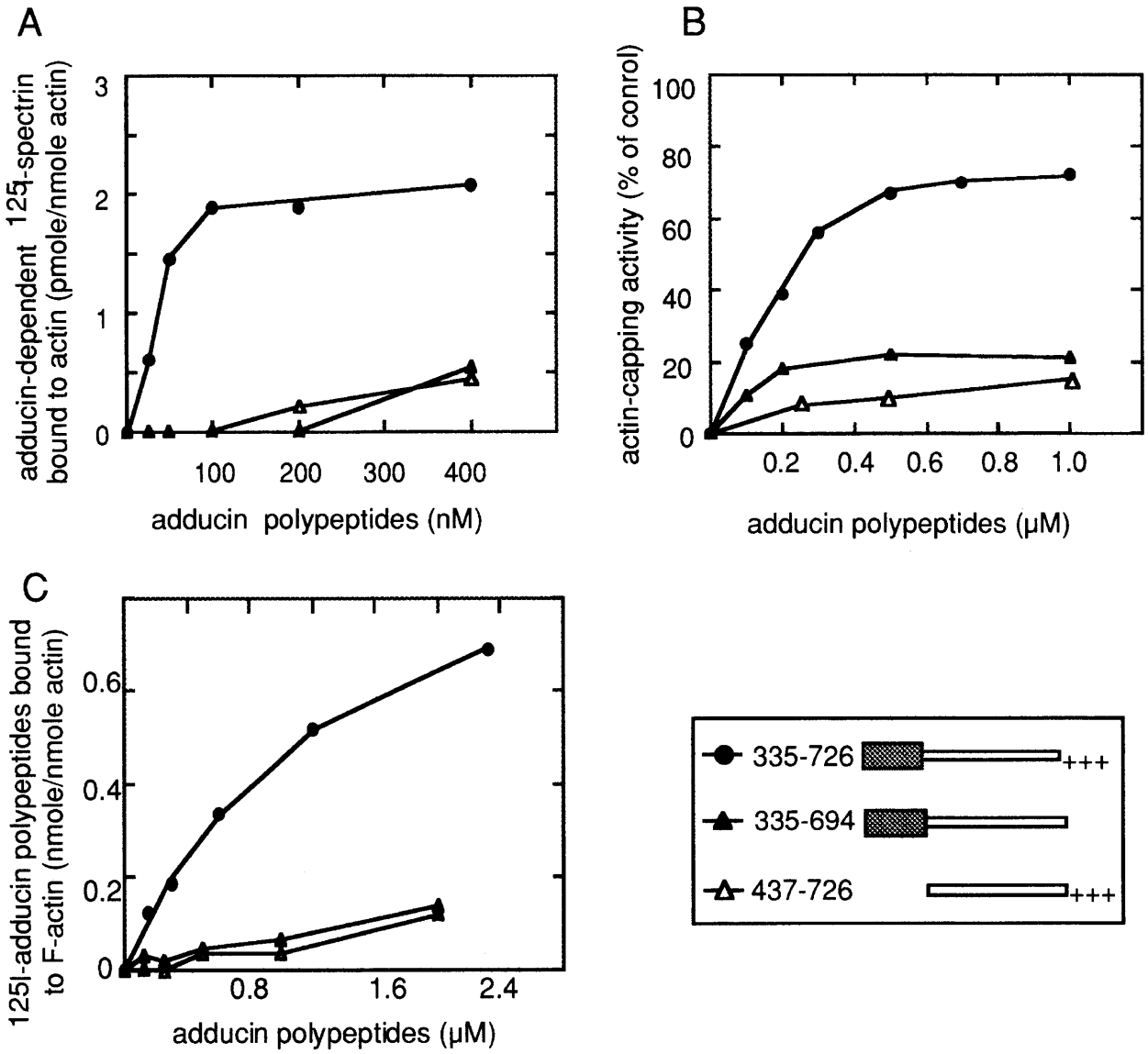

Figure 4. Comparison among β-adducin polypeptides in spectrin-recruiting, actin-capping and actin-binding activities. (*A*) Spectrin-recruiting activity was examined in an actin cosedimentation assay. ^{125}I-labeled spectrin and increasing concentration of adducin were incubated with F-actin immobilized on microsphere beads as described [29]. (*B*) Actin-capping activity was examined in an actin polymerization assay using pyrene-labeled actin. The degree of capping was expressed as percentage of inhibition of F-actin elongation. (*C*) ^{125}I-labeled adducin polypeptides with serial dilution were incubated with immobilized actin.

necessity of this domain in all adducin activities. An alternative explanation for the critical role of the MARCKS-related domain is its potential contribution to adducin folding. This possibility is not likely, because adducin tail is mostly in random coil configurations [25]. The polybasic nature and the phosphorylation effects (see below) imply that ionic interactions appear to play a major role in adducin association with spectrin-actin. Indeed, spectrin-recruiting activity of adducin was almost completely abolished in the presence of 150 mM NaCl [X. Li, unpublished data]. Since the MARCKS-related domains are highly conserved in all adducin subunits, the results suggest a general principle that this domain mediates contact between adducin and spectrin-actin via interactions that include electrostatic bonds.

Negatively charged residues (aspartate or glutamate) are exposed on the lateral surface of rabbit skeletal actin in positions 1–4, 24, 25, 99, 100, 360, 361, 363 and 364, whereas residues 167, 288 and 292 are located at

the fast-growing ends of actin filaments [42, 43]. The negatively charged residues exposed on the lateral surface of actin have been implicated in association with a positively charged lysine-rich loop in the head domain of myosin [44, 45] and comprise a possible actin-myosin interface [42, 43].

The neck domain is required for adducin activities and self-associates to form oligomers. The neck domain itself had no detectable spectrin-recruiting, actin-capping or actin-binding activities, indicating that the neck domain is not sufficient for any of these functions. However, deletion of the neck domain from β adducin 335–726 resulted in almost complete loss of spectrin-recruiting, actin-binding and actin-capping activities [29]. These findings suggest that the neck domain is necessary but not sufficient in itself for interactions of adducin with spectrin and actin.

Adducin is known to associate into dimers and tetramers [18, 25, 30]. Contribution of the domains in oligomerization of adducin has been evaluated by chemical cross-linking experiments. The neck domain and any polypeptides containing the neck domain are cross-linked into oligomers [29]. The major form of oligomers is a dimer, but trimers, tetramers and higher oligomers are also evident. In contrast, the constructs lacking a neck domain are not cross-linked, and remain as monomers. The cross-linking reaction reflects specific protein-protein interactions, because the majority of the cross-linked products are abolished in the presence of 6 M urea [29]. It is noteworthy that native erythrocyte adducin forms tetramers as the major cross-linked products [25, 29], suggesting that additional oligomerization sites must exist which further connect α and β adducin dimers into heterooligomers.

Within the neck domain (aa335–436) of β adducin, the most conserved stretch encompasses residues 357–387 (fig. 5). This region is conserved among human α and β adducin subunits [22], rat γ adducin [23] and *Drosophila* Hts [46; for details see below]. This region has the highest probability to form amphiphilic α helices in adducin. An amphiphilic helix, with hydrophilic residues distributed along one side of the helix with hydrophobic residues on the other side, is an important feature of many coiled-coil structures which mediate protein-protein interactions [47].

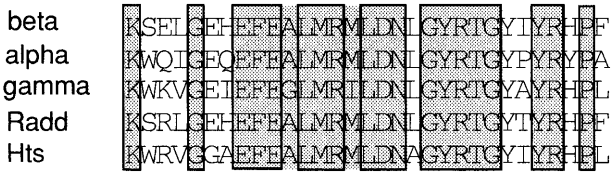

Figure 5. A conserved region in the neck domains of adducin and adducin homologues. Human adducin subunits (α, β, γ), rat γ adducin (Radd) and *Drosophila* adducin-like protein (Hts) are aligned.

Importance of the conserved stretch in neck domain for oligomerization of adducin has been further demonstrated in another series of experiments [48]. A methionine residue at 369 of β adducin was mutated to proline. The mutated residue is in the middle of the highly conserved region of the adducin neck (fig. 5), thus the mutant will theoretically break or distort potential α helices in that region. Purified protein of both wild-type and the M369P mutant form of β 335–726 were analyzed by chemical cross-linking experiments. Quantitative results showed that a maximum of 15% of M369P mutants are cross-linked, whereas 60% of wild-type protein can be cross-linked. This significant decrease in oligomerization activity in the M369P mutant may be caused by disruption of the α-helical structure by the presence of the proline residue, which suggests that the α-helical structure plays a critical role in oligomerization of the neck domain, probably through formation of a coiled-coil motif.

Adducin is unusual in its ability to associate with both the sides and ends of actin filaments. Tensin is another protein with multiple actin-binding activities and caps F-actin, binds along the sides and bundles the filaments [49]. However, the actin-binding and actin-capping activities of tensin are located in different domains. The unique feature of adducin is that the same regions, the neck domain (for oligomerization) and the MARCKS-related domain, are required for actin capping, direct actin binding and spectrin recruitment.

Regulation of adducin functions by phosphorylation and Ca^{2+}-calmodulin. Adducin is a substrate for various protein kinases, and its activities are regulated by phosphorylation. Adducin is phosphorylated by PKC in vitro and in vivo [33, 50–52]. PKC phosphorylation of adducin inhibits both the actin-capping and spectrin-recruiting activities [33]. Cyclic AMP (cAMP)-dependent protein kinase (PKA) also phosphorylates adducin in vitro and downregulates actin-binding and spectrin-recruiting activities [51, 52]. Ser726 and Ser713 in the MARCKS-related domains of α and β adducin, respectively, are the major phosphorylation sites common for PKA and PKC. PKA, in addition, phosphorylates α adducin at Ser408, Ser436 and Ser481 [52] (fig. 2). Rho-associated kinase (Rho-kinase) phosphorylates α adducin at Thr445 and Thr480 [53], and enhances adducin-actin interactions [54]. Although myosin phosphatase, which itself is a downstream target of Rho-kinase, is the only adducin phosphatase identified so far [54], other types of phosphatases have yet to be tested.

Ca^{2+}-calmodulin inhibits adducin activities in both actin-capping and spectrin-recruiting aspects as well [14, 19]. Interestingly, the major calmodulin binding site in adducin is the MARCKS-related domain, which contains the common phosphorylation site for PKA and PKC [52].

A model of the membrane skeleton assembly

Adducin potentially fulfills several critical tasks in the assembly of the spectrin-actin network such as recruiting spectrin to fast-growing ends of actin filaments and capping the ends with high affinity in the presence of spectrin. Previous reports showed that neither spectrin-actin-protein 4.1 complexes nor spectrin-actin-adducin complexes were able to form the network structure of the membrane skeleton [17, 20]. The minimal factors to form the short actin filaments appear to be spectrin, adducin, tropomyosin and tropomodulin. Spectrin-adducin caps the fast-growing ends of actin filaments, whereas tropomodulin-tropomyosin caps the slow-growing ends of filaments. Once the short actin filaments are precisely formed and maintained, spectrin molecules can bind to the filaments through low-affinity spectrin-actin association and are stabilized by high-affinity spectrin-protein 4.1 interactions.

The stoichiometry of membrane skeletal proteins in erythrocytes provides insights into assembly of the spectrin-actin network. On average each erythrocyte contains 30,000 short actin filaments, 100,000 spectrin tetramers (containing 200,000 copies of actin binding domains), 200,000 copies of protein 4.1, 15,000 copies of adducin tetramers, 30,000 copies of tropomodulin and 70,000 copies of tropomyosin [1]. It is likely that the number of adducin tetramers in native membranes is greater than in isolated membranes because adducin is lost during hypotonic lysis of erythrocytes in the absence of magnesium ion [34]. These numbers, adjusted to include loss of adducin, are consistent with a model where each junctional complex includes 6 spectrins, 6 copies of protein 4.1, 1 adducin tetramer, 1 tropomodulin and 2 tropomyosins. However, differences in the stoichiometry may exist in different cell types.

The in vitro activities of adducin imply that an important role of adducin in cells is to form a complex with the fast-growing ends of actin filaments that recruits spectrin and prevents addition or loss of actin subunits. Adducin is the first example of an actin-capping protein that recruits other proteins to actin filament ends, and could represent a new class of assembly factor with the function of integrating actin into other cell structures. The number of spectrin molecules recruited per adducin is not yet known. However, a possibility consistent with available data is that one spectrin tetramer is stabilized for each adducin dimer, and two spectrin molecules are associated with adducin tetramers. In this case, spectrin tetramers, which contain two actin-binding domains, would form a one-dimensional chain which could be the structural precursor to the two-dimensional network of the mature erythrocyte membrane skeleton [4–6]. Additional spectrin molecules associated with lateral sites along actin filaments may be stabilized by protein 4.1, which is present in approximately one copy per spectrin dimer. Actin filaments in erythrocyte membrane skeletons are likely to contain tropomyosin, associated along the filament groove [13], tropomodulin, which binds tropomyosin and caps the slow-growing ends of actin filaments [15], as well as dematin [55]. It will be of interest to evaluate association of adducin with actin complexed with these accessory proteins. A prediction is that adducin will be excluded from sites on actin filaments coated with tropomyosin, and will be confined to filament ends [7].

Spectrin and ankyrin are incorporated into membrane skeletal structure only after major integral proteins are expressed [56], implying the potential linear linkage of spectrin-actin complexes is attached to the membrane by association of spectrin with integral proteins through adapter proteins such as ankyrin. Each linear assembly of spectrin-actin on the membrane is likely to connect to similar liner linkages in nearby areas on the membrane and generate two-dimensional networks. Protein 4.1 (two copies per spectrin) promotes association of additional spectrin with actin, and thereby could cross-link a one-dimensional structure into a two-dimensional network in the second step of the assembly. This two-step assembly reduces the possibilities that any linear linkages of spectrin-actin extend into the cytoplasm and form a three-dimensional mass.

Cellular localization and in vivo activities of adducin

Adducin is localized at spectrin-actin junctions in mature erythrocytes [57], and is expressed in early erythropoiesis when the spectrin-actin network is forming [58]. Adducin and spectrin are both concentrated at sites of cell-cell contact in epithelial tissues, and at dendritic spines of cultured neurons [33, 59]. Expression of a dominant-negative form of spectrin that inhibits assembly of spectrin tetramers results in loss of epithelial polarity and disassociation of adducin from the plasma membrane [60].

Adducin is expressed at high levels in brain and is identified as a constituent of synaptic structures [30, 61]. Adducin binds to tetanus and botulinum toxins in a ganglioside-dependent manner [62], and binds to Rabphilin-3A, a synaptic vesicle protein that regulates neurotransmitter release [63, 64]. Adducin and spectrin are both concentrated at dendritic spines and growth

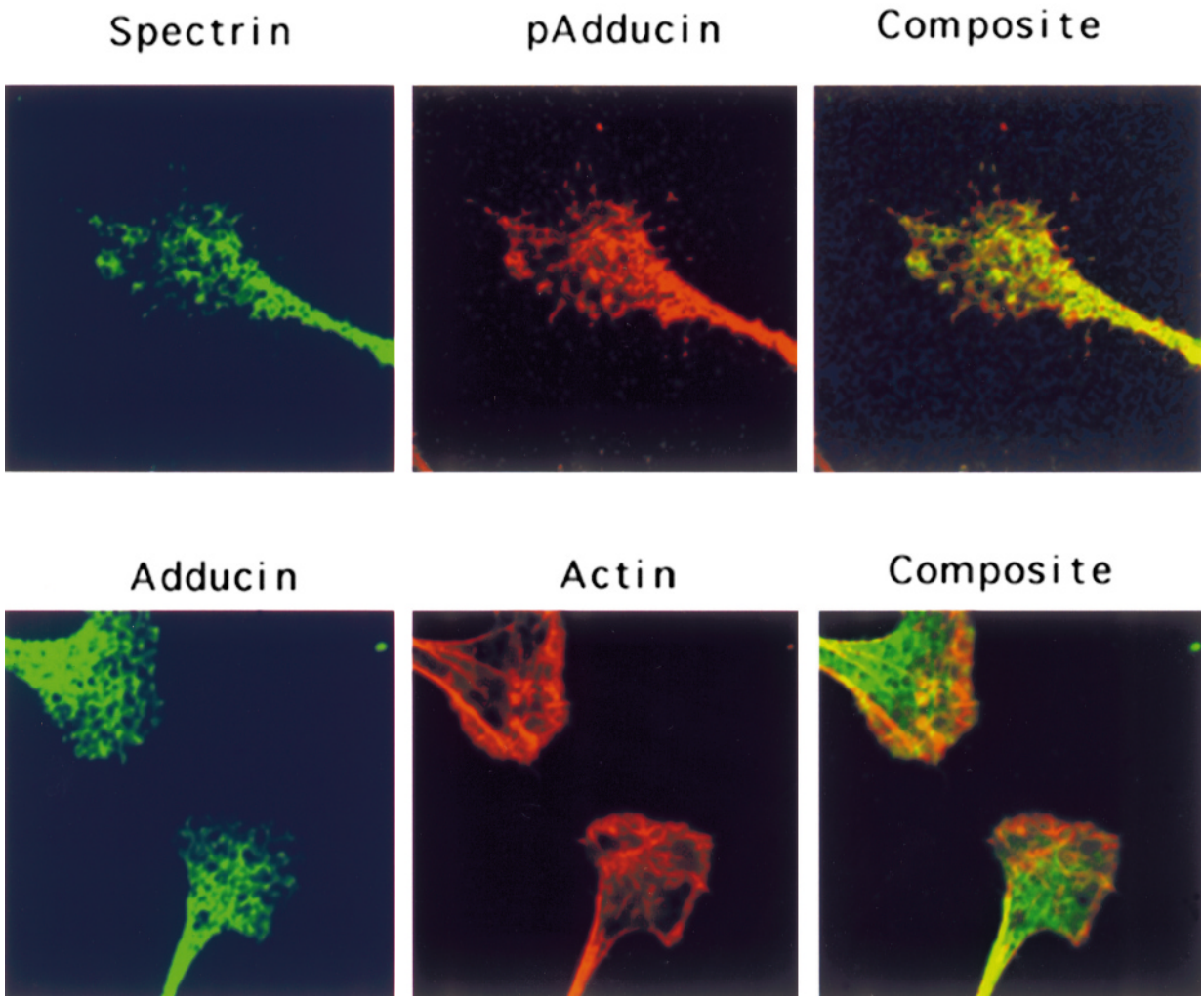

Figure 6. Localization of spectrin, adducin and phosphoadducin in axonal growth cone. Dorsal root ganglion neurons were stained with anti-βG-spectrin monoclonal (5 μg/ml), anti-phosphoadducin polyclonal (3.5 μg/ml) and anti-α adducin polyclonal (3 μg/ml) antibodies, and were visualized with FITC- or TRITC-conjugated goat secondary antibodies (1.5 μg/ml each). F-actin was visualized by rhodamine-phalloidin. Composite images show colocalization of the proteins (yellow).

cones of cultured neurons [33 and fig. 6]. This pattern of localization suggests the possibility that adducin plays some role in the dynamic regulation of spectrin assembly at synapses. Most recently, a β adducin knockout mouse has been reported [65]. The mouse shows decreased deformability of erythrocytes, resulting in anemia and deficiency of α adducin in erythrocytes, which is consistent with the proposed function of adducin in the membrane skeleton. It will be of particular interest to determine if the β adducin $(-/-)$ mouse has any neurological abnormalities such as impaired learning ability and memory.

In vivo studies correlate with the previous findings that adducin activities can be regulated by PKC phosphorylation. Phorbol ester 12-*o*-tetradecanoylphorbol-13-acetate (TPA) activation of PKC induces redistribution of adducin away from cell contact sites of epithelial cells [23, 59]. Furthermore, adducin is phosphorylated by PKC at high levels in renal carcinomas, and this phosphorylation correlates with changes in the subcellular distribution of adducin [66].

Adducin phosphorylated at the major PKC site is concentrated in dendritic spines of hippocampal neurons [33] and in lamellipodia of platelets (fig. 7) and fibroblasts, both of which contain dynamic actin structures. Overexpression of a PKC-resistant mutant of α adducin (Ser716 and Ser726 are substituted by Ala) but not wild-type adducin interferes with migration of fibroblasts [Y. Matsuoka, unpublished data], suggesting that adducin is a part of the cell motility machinery. In

addition, phosphorylation of adducin by Rho-kinase has been shown to enhance the adducin/actin interaction [54], and Thr445 and Thr480 of α adducin are identified as the phosphorylation sites [53]. α adducin phosphorylated at Thr445 accumulates in the membrane ruffling area and the leading edge of Madin-Darby canine kidney (MDCK) epithelial cells treated with TPA and hepatocyte growth factor (or scatter factor). Intriguingly, overexpression of an α adducin mutant, which is substituted Thr445 and Thr480 by Ala, also inhibits the TPA-induced membrane ruffling and wound-induced cell migration [53]. These results indicate that adducin is a downstream effector of Rho-kinase as well as PKC, and that phosphorylation of adducin by these kinases plays crucial roles in the regulation of membrane ruffling and cell motility.

Adducin polymorphisms have been proposed to account for some types of hypertension both in rats and humans [67, 68]. In Milan hypertensive strain (MHS) rats, two point mutations α F361Y and β Q529R are associated with hypertension in genetic crosses of MHS and normal rats. One group has reported that polymorphism of α adducin (G460W) is significantly linked to hypertension and salt sensitivity in humans [69]. However, other genetic studies, studying entirely disparate populations, have not confirmed that the α-adducin G460W polymorphism is related to blood pressure in humans [70–73].

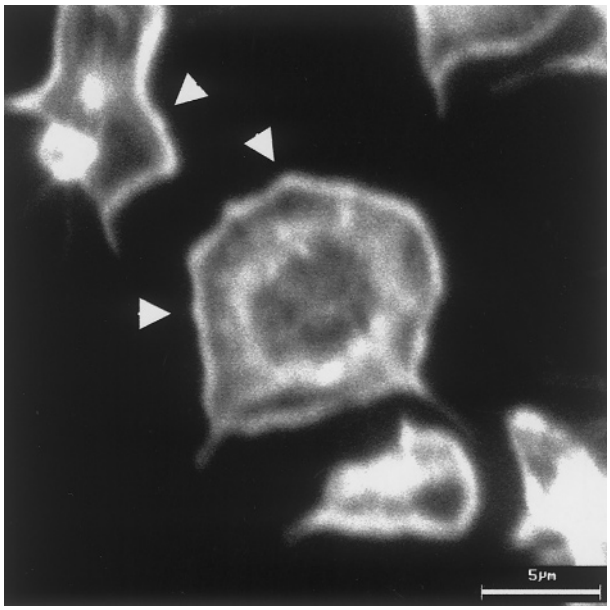

Figure 7. Localization of phosphoadducin in activated platelets. Human platelets were activated on a cover glass and stained with anti-phosphoadducin antibody (3.5 μg/ml). Phosphoadducin was accumulated in the lamellipodia (arrowheads).

Adducin-like genes in *Drosophila* and *Caenorhabditis elegans*

Genetic studies of *Drosophila* have identified an adducin homologue that is required for oogenesis [46]. The ovary of the fly contains multiple egg chambers. Each egg chamber consists of a 16-cell structure, including 1 oocyte and 15 nurse cells. The 16 cells are interconnected by ring canals, which are special cytoplasmic bridges that transport cellular components from nurse cells to the oocyte. Female sterile mutations featured with fewer nurse cells and a lack of oocytes have led to the cloning of a new *Drosophila* gene. The gene was named *Hu-li Tai Shao* or Hts ('too little nursing' in Chinese), and was identified as an adducin-like gene. Hts encodes a protein of 1156 amino acids, with the N-terminal 800 residues 37% identical and 56% similar to both α and β adducin [46, 74]. The region corresponding to the head and neck domains of human adducin is highly conserved. However, the last 160 residues of adducin (second half of the tail region), including the MARCKS-related domain, show little correspondence to Hts. In addition, Hts has 400 extra residues at the C-terminus, suggesting specialized functions for the adducin-like gene in *Drosophila*.

Flies with a mutation in the Hts are sterile, displaying abnormal ring canals with a lack of associated actin rings [46]. A simple explanation is that Hts is required to bind to ring canal rims and anchor actin filaments. Hts, as well as spectrin and ankyrin, is also found in the fusome, a large cytoplasmic structure during *Drosophila* oogenesis [75]. Hts mutants completely eliminate fusomes, a phenotype similar to that of α-spectrin deficiency [75, 76]. The role of Hts in germline cell division is reinforced by the finding that the Hts mutant disrupts polarized microtubule-based transport that is involved in oocyte differentiation [77].

An adducin homologue has also been found in *C. elegans* [78]. *C. elegans* has homologues of spectrin and ankyrin as well, providing opportunities to study adducin and the membrane skeleton in another genetic system. On the other hand, no adducin genes are found in yeast, and neither are other spectrin skeletal proteins. It is consistent with the previous notion that the spectrin-based skeleton exerts functions in multicellular organisms [2].

Concluding remarks and future directions

The MARCKS-related domain of adducin is required for all adducin functions analyzed, such as spectrin-recruiting, actin-capping and actin-binding activity. Based on current information, it appears that the MARCKS-related domain mediates direct contact between adducin and spectrin-actin via ionic interactions which are in-

hibited by PKC phosphorylation at the MARCKS-related domain. The finding of ionic interaction between adducin and actin raises an intriguing possibility that adducin may compete for the negative residues of actin with tropomyosin and/or myosin. The negatively charged residues exposed on the lateral sides of actin filaments may include Asp or Glu residues at positions 1–4, 24, 25, 99, 100, 360, 361, 363 and 364 [42, 43]. The tropomyosin-actin interaction is mediated by the charged residues on the sides of actin filaments [79]. Potential competition of tropomyosin with adducin for binding to the sides of actin will further direct adducin to the ends of actin, which has significance in determining the stoichiometry of proteins assembled at the junctional complex of erythrocytes. It will be of interest to test the competition of tropomyosin and adducin in the future by examining actin-binding activity in the presence of tropomyosin.

An interesting issue is a role for adducin in myosin-based cell motility. We have found that adducin inhibits actin-activated adenosine triphosphatase (ATPase) activity of myosin subfragment-1 (S1) in a phosphorylation-dependent manner. Conversely, S1 competes with adducin for binding to actin [80]. These results imply that by regulating the accessibility of actin to myosin, adducin potentially serves as a molecular switch for the myosin-based cell motility.

Since adducin locates at the spectrin-actin junction, a strategic site for control of cytoskeleton-membrane skeleton interactions, and is a downstream effector of several signaling pathways, adducin may be an integrated component of the machinery that converts extracellular stimuli into cell shape change or cell movement as an output.

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