A review of actin binding proteins: new perspectives

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Abstract Actin binding proteins (ABPs) have been considered components of the cytoskeleton, which gives structure and allows mobility of the cell. The complex dynamic properties of the actin cytoskeleton are regulated at multiple levels by a variety of proteins that control actin polymerization, severing of actin filaments and cross-linking of actin filaments into networks, which may be used by molecular motors. Proteins that cross-link F-actin are important for the maintenance of the viscoelastic properties of the cytoplasm and for the integrity of plasma membrane-associated macromolecules. Most of these F-actin cross-linking proteins have an actin-binding domain homologous to calponin. In addition, some of them have been considered scaffolds. Through the years, several research groups have found different proteins that interact with ABPs; however, the effect of these interactions on ABPs remains mostly unknown. In addition to organize the cytoskeletal structure, recent data indicate that ABPs can also migrate to the nucleus. This fact is in agreement and could be relevant to the recently found role that actin might play in nuclear function. Recent data and analysis of published results have also indicated that scaffold proteins like filamin A (FLNa) may be processed by proteolysis and that the degradation products generated by this reaction may play a role as signaling molecules, integrating nuclear and cytosolic pathways. Some of the relevant information in this area is reviewed here.

Abbreviations

ABPs	Actin binding proteins
ABD	Actin-binding domain
CH domains	Calponin homology domains
FLNa	Filamin A
SIPPs	Signaling integrator proteolytic peptides

Actin in the nucleus

The actin cytoskeleton of eukaryotic cells plays a central role in many cell functions such as cell shape, cell division, motility, contraction, adhesion, phagocytosis, protein sorting and signal transduction. The complex and dynamic properties of the actin cytoskeleton are regulated at multiple levels by a variety of proteins that control actin polymerization, severing of actin filaments and cross-linking of actin filaments into networks or bundles. Apart from being a fundamental component of cytoskeleton, actin has been found in the cellular nucleus carrying out different functions. Many studies have established the presence of actin in the nucleus and have shown that its functions are as diverse here as the ones found in the cytoplasm. Possible roles for nuclear actin include contribution to the organization of chromatin remodeling complexes, RNA processing, or regulation of DNAse I function [1, 2]. In addition, actin plays a direct role in transcription by RNA polymerases I, II and III [3–6]. Until now the mechanism by which actin migrates to the nucleus is unknown. In this

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regard, myosin has been found with actin in the nucleus, physically associated with both RNA polymerase I holo-enzyme and the ribosomal genes [3]. The ability of myosin to bind actin is shared by other actin binding proteins (ABPs), that contain actin-binding domains (ABD), and these ABPs may localized to the nucleus too. Filamentous actin (F-actin) cross-linker proteins are very abundant in normal cells. The possibility exists, therefore, that these proteins migrate to the nucleus together with actin by means of these ABDs and in this way they may transport actin to the nucleus. In this regard, however, it is worthwhile mentioning that there is a growing body of evidence indicating that nuclear actin is not in a conventional form but rather it is present as short oligomers. In this sense, ABPs would not provide a nucleating unit around which an F-actin network could be organized; especially in the cases where ABPs have already been digested (see below). Therefore, nuclear actin, alone or in complex with ABPs, would provide a function different from the structural one found in the cytoplasm [7, 8].

Actin binding proteins with CH domains

There is a great diversity of proteins that bind actin, which are important contributors to cell structure. These proteins are important in maintaining the viscoelastic properties of the cytoplasm, to protect the integrity of the plasmatic membrane-associated macromolecules and to serve as scaffolds. In eukaryotic cells, there are diverse F-actin cross-linking proteins. Many of these proteins have actin binding domains composed of two calponin homology

(CH) regions. The CH domain is a protein domain of ∼110 residues found in cytoskeletal and signal-transduction proteins [9]. Two CH domains in tandem constitute the actin-binding region of members of several protein families that cross-link actin filaments forming networks or bundles. These families include proteins such as spectrin, α-actinin, dystrophin and FLNa (Fig. 1). The surface of the repeats of these proteins may serve as docking sites for cytoskeletal and signal transduction proteins. For a long time the idea existed that these ABPs were exclusively found associated to the cytoskeleton in the cytoplasm, but evidence has arisen recently that demonstrates that they are also found in the nucleus carrying out diverse functions.

ABPs in the nucleus: a reality

The demonstration of the location of a protein that binds actin in the nucleus was published for the first time in 1987 [10]. Thereafter, other actin-binding proteins have been located in the nucleus (Table 1).

α-actinin interacts with more than 29 components in the cell [11], and there are different isoforms of this protein, like α-actinin-4, that cross-links actin filaments and reorganizes the cytoskeleton for cell movement. Nevertheless, it has also been observed that α-actinin-4 is present in the nucleus of a particular population of breast cancer cells. However, from the deduced aminoacid sequence, it seems that actinin-4 does not possess any apparent nuclear localization signal [12]. Likewise, Pinaev's research group found that the α-actinin-4 co-localizes to the nucleus with p65/RelA subunit of NF-kappa B [13]. In addition,

Fig. 1 Schematic representation of the different proteins that bind actin. Some similarities in the structure can be appreciated. These proteins are formed by repetitions, (alpha or beta) of approximately 100 aminoacids. The CH domains are present in most of these proteins
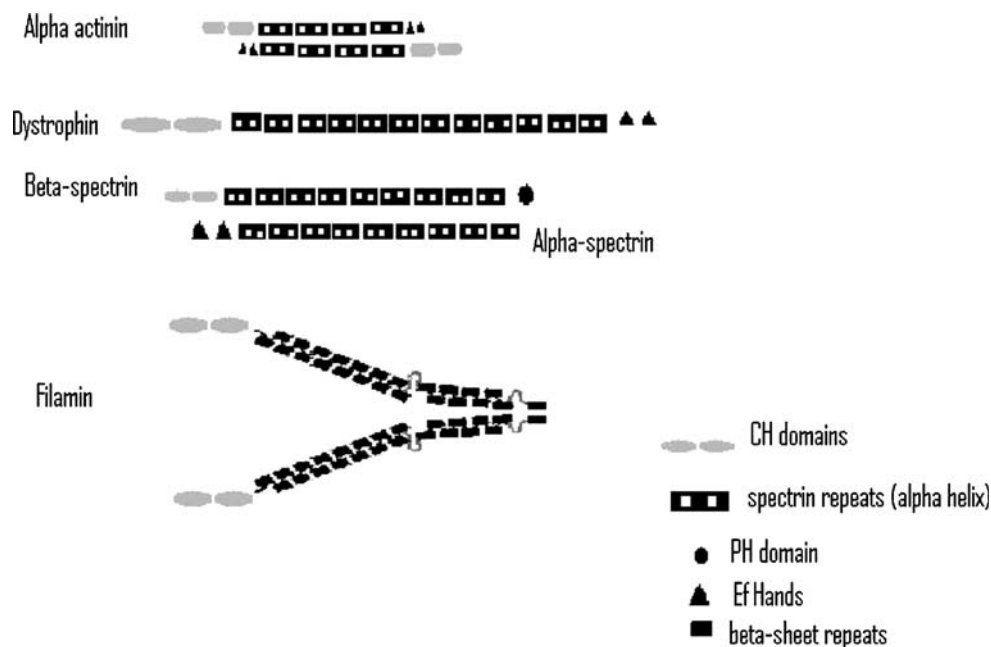
Table 1 Actin binding proteins with CH domains found in the nucleus

Actin binding proteins	References
Alpha actinin-4	[12–14]
α-Spectrin II	[16–18, 20]
β-spectrin II	[19]
Filamin A	[23, 24, 27]
Dystrophin	[31]
Vav	[32]
Nesprin-2	[35]
L-Plastin	[36]

α-actinin 4 potentiates myocyte enhancer factor-2 transcription activity by antagonizing histone deacetylase 7 [14]. These results demonstrate that this 'cytosol-associated cytoskeletal protein' has important functions in the nucleus.

Another cytoskeletal F-actin cross-linking protein localized to the nucleus is spectrin. This protein has diverse isoforms. Spectrin was also detected at the nuclear envelope, as well as in the intranuclear granules within liver cells [15].

Strong evidence exists indicating that α-SpecII isoform of spectrin plays a role in DNA repair, possibly acting as a scaffold for DNA repair proteins [16–18]. Other isoform of spectrin, β-SpecII, is present in the nucleus and interact with nuclear proteins. β-SpecII associates with Smad proteins in the cytoplasm and once associated, these proteins can translocate to the nucleus of liver cells [19].

Deepa M. Sridharan et al. have observed that nonerythroid ∝-spectrin IIΣ* (∝SpIIΣ*) associates with five different groups of functionally important proteins in the nucleus of normal human cells: structural proteins, proteins involved in DNA repair, chromatin remodeling proteins, Fanconi anemia proteins and proteins which play a role in transcription and processing of RNA [20].

Another cytoskeletal-associated protein that does not belong to the spectrin family, but has also been located to the nucleus is filamin A (FLNa). Filamins are homodimeric F-actin cross-linkers, with a high molecular weight (280 kDa for FLNa), which organize actin filaments into parallel arrays or three-dimensional webs, linking them to cellular membrane [21]. This protein anchors a variety of transmembrane proteins to the actin cytoskeleton and provides a scaffold for many cytoplasmic and signaling molecules [22].

Ozanne and Loy observed that the C-terminal region of the FLNa could translocate to the nucleus together with the androgen receptor (AR) [23, 24]. The C-terminal region of FLNa is a 100-kDa polypeptide. This polypeptide can be generated by the action of calpain when FLNa is dephosphorylated in Ser 2152 [25, 26]. FLNa can migrate with other proteins to the nucleus, for example Protein FOXC1 [27]. Although in this case, it remains to be established whether full length FLNa or a proteolysis fragment of it is the migrating element. Furthermore, FLNa regulates the subcellular localization of Smad2 and PEBP2/CBF, two transcription factors that are usually found in the cytoplasm [28, 29].

There is a growing body of evidence implicating FLNa in the regulation of nuclear functions including DNA repair [30].

Another ABP with a CH domain is Dystrophin 71 (Dp71), which was reported to be found in the nucleus of PC12, cells an established cell line derived from a rat pheochromocytoma [31].

At this point the question arises as to whether all these ABPs could migrate to the nucleus associated with actin. In this regard, it is worthwhile mentioning that FLNa was localized in the nucleus by Fred Berry et al. utilizing an antibody against the N-terminal actin-binding region of the protein [27].

It is now clear that ABPs and actin perform a variety of functions in the nucleus. How these proteins are mobilized between the nucleus and the cytoplasm and whether they control nuclear processes on their own or in association with each other, remains as open questions. Although there is no final evidence indicating that an association of this kind can play a part in the mobilization of actin to the nucleus, this possibility cannot be ruled out at the moment since different ABPs with CH domain have been found in the nucleus. For example, vav, a 95 kDa CH-containing GDP/GTP exchange proto-oncogene product expressed exclusively in hematopoietic cells was found to bind to Heterogeneous Nuclear Ribonucleoprotein (hnRNP) C through a region in the C-terminal domain of the protein. The interaction was regulated by poly(U) RNA and was proposed to play a part in pre-mRNA synthesis and mRNA export [32]. Whether vav was bound to hnRNP C alone or in complex with actin through its CH domain remains as an open question. However, it is worthwhile mentioning that more recent results showed that actin and hnRNP U cooperate for productive transcription by RNA polymerase II [33]. In this case hnRNP U also bound actin through a short amino acid sequence in the C-terminal region. In addition, Laury-Kleintop et al. recently reported that hnRNP K form a signaling complex with calponin that may be involved in growth-stimulated post-transcriptional regulation. In this case the complex was found mainly in a cytoplasmic location [34].

How can the ABPs arrive at the nucleus?

Since it was known that ABPs could be localized to the nucleus, the search started for a possible mechanism for

their mobilization. There has not been final evidence indicating whether full-length ABPs are mobilized within the cell and to the nucleus or only fragments of them, probably produced by protease action, are required to fulfill the function. Previous studies have demonstrated that in this case the susceptibility of some of ABPs can be regulated by phosphorylation as in the case of the FLNa or alpha II spectrin [25, 26, 37]. In the case of FLNa, two major peptides are generated after proteolysis, corresponding to the C-terminal 100 kDa and N-terminal 180 kDa regions of the molecule. In this regard, Ozanne and Loy found that the C-terminal region of FLNa could be translocated to the nucleus associated to the AR [23, 24]. Previous studies demonstrated that the generation of this C-terminal fragment of FLNa could be prevented by the phosphorylation of Ser 2152 by PKA [25, 26].

The results of Ozanne and Loy indicated that at least one region of the protein could carry out a specific function, i.e. mobilization of the AR to the nucleus. Most of the ABPs are also susceptible to degradation by caspases and could be also the target or other proteases.

We have recently proposed that proteolysis of scaffold proteins and other possible targets could provide a general mechanism to integrate cellular pathway within the cell. The protein fragments produced by proteolysis could function as "Signaling Integrator Proteolytic Peptide" (SIPPs) (Fig. 2), and their generation could be regulated by a phosphorylation/dephosphorylation processes [38]. We anticipate that many of the ABPs could also participate in

nuclear and cytoplasmic routs by these means. Thus, it is possible that ABP SIPPs alone or in combination with actin could serve as nuclear scaffolds to proteins involved in DNA repair or chromatin complex formation.

The study of the nuclear ABPs could probably require the analysis of the proteolytic fragments generated from them that could represent the true functional peptides.

Fig. 2 Schematic representation of SIPPs generation. SIPPs from ABPs or other targets can translocate to the nucleus together with different proteins

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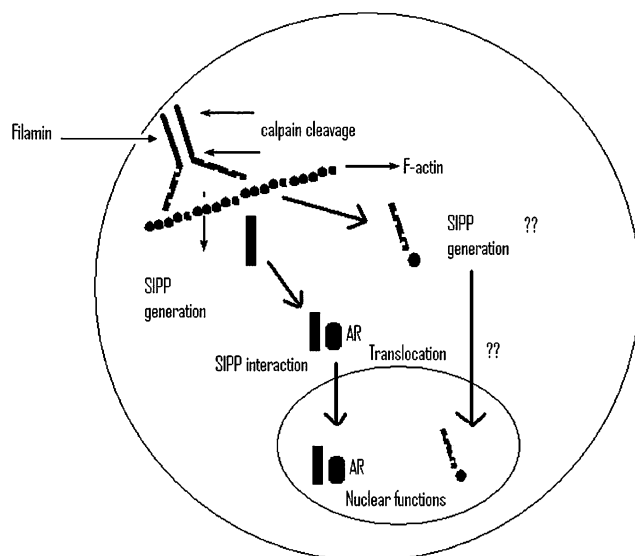16. McMahon LW, Walsh CE, Lambert MW (1999) Human alpha spectrin II and the Fanconi anemia proteins FANCA and FANCC

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