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REVIEW ARTICLE

Significance of Actin Bundling Protein In Hearing: An Update

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ABSTRACT

Noise-induced hearing loss (NIHL) is caused due to recreational settings. Data reported that 16% of hearing impairment is due to occupational exposure at workplaces. NIHL gradually develops after a long exposure to 85dB or more. The inner cochlear hair cells stereocilia (mechanosensory cells), deranged due to loud noise and disrupts structural integrity. The structural actin bundling proteins espin, fimbrin, villin and fascin involved in bundling of the actin filaments (F-actin) of steriocilia. The key role of individual protein in cytoskeletal actin bundling of stereocilia is responsible for lengthening of stereocilia and hearing acuity.

This review tried to enlighten on the actin bundling proteins which play a significant role in the stereocilial lengthening and perception of sound.

Keywords: *Actin bundling proteins, Espin, Fascin, Fimbrin, Inner Hair Cells, Outer Hair Cells, Stereocilia, Villin*

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INTRODUCTION

Worldwide noise is a major problem due to heavy traffic, industries, construction and mining activities etc. 16% of hearing impairment in adults is assign to occupational noise, ranging from 7% to 21% in various subregions reported by World Health Organization (WHO) [1]. Noise upto 85dB is tolerable but above this for long exposure cause Noise Induced Hearing Loss (NIHL). NIHL is a principal occupational disease, gradually develop and loss of high frequency hearing sensitivity over a time. It can be temporary like exposure to impulse loud noise, disappear in 16 to 48 hours later, or permanent. Profound hearing loss is the deafness where affected person only able to hear loud noises. Later stages of NIHL, goes critical and the person looses even understanding towards human speech [2].

The sensorineural hearing loss is the permanent, most common types of NIHL, functional loss in inner ear, damages in auditory nerve pathway and hair cells. The innermost part of the ear is known as inner ear, involved in the hearing sequence. The sound wave first enter the outer ear, hits ear drum, vibrates to the three bones of middle ear, Incus, malleus and stapes with same frequency, follows the cochlear fluid ripple [3]. As on the apical surface of basilar membrane the hair cells are arranged in chain sequence. The inner hair (IHC) transforms mechanical force of sound into electrical impulses of hearing. Communicate with nerve fiber that comprises the auditory nerve leading to brain. While the outer hair cell (OHC) actively and rapidly elongate in response to voltage change in cell membrane. The functioning is called as electromotility. Each OHC amplifies a part of signal so that all sounds become amplified and the amplified sound then recognize by the IHC and sent to the brain [4]. Although the OHCs and IHCs are the mechanoreceptors dispensing similar general characteristics such as organization of stereocilia, linkage system and the series of changes of the stereociliary height along the length of cochlea. A small structure organized on hair cells, the stereocilia are the mechanosensory cells, arranged in a row of similar and

disimilar length produces staircase assemblies. It has a huge inference on hearing because it contains the channels that convert vibration and motion into electric current, alters the cross-membrane potential, and is essential for hearing [5].

The stereocilia grows remarkably different in length that emerges from the electron dense patches below the plasma membrane. Multiple classes of proteins are involved in the different functions of hearing. The lengthening of stereocilia play a major role in the hearing ability, as some proteins are responsible for lengthening known as villin, myosin IIIa and espin. The protein espin also has a crucial role in the lengthening of stereocilia, also the protein called villin produces measurable lengthening [5]. The other protein Myosin IIIa, a actin based motor protein [6], the protein also seen in the lengthening of mechanosensory stereocilia. Scientist reported that an ankyrin repeat protein espin1 containing isoforms, colocalizes with myosin IIIa at stereocilia tips and interconnect with a unique conserved domain of myosin IIIa, shown that combined expression of both these proteins causes greater elongation of stereocilia, compared with an individual expression of either myosin IIIa or espin 1 alone [7].

The stereocilia, visible during early development and their lengths are maintained at fixed heights for the lifetime of the organism [7], filled with parallel actin filaments (F-actin). Actin is abundant protein used by cells and actin filaments for cross linking involved cell structure division, motility, adhesion and signalling. Globular protein actin (G-actin) an individual molecule, having 375 amino acids [8]. Including three isoforms in vertebrates the α-isoforms of skeletal, cardiac and smooth muscles, and β and ϒ-isoforms expressed in non-muscles and muscle cells. The isoforms differ by only few amino acids, with most variations toward the N-terminus.

In stereocilia G-actin structure having a pointed end (-) and a barbed end(+), monomers polymerize to form actin filaments (F-actin) the crystal structures resolve in the ATP-bound form.. The polymerization begins with the spontaneous nucleation of monomer (G-actin) to form a trimer. It is reversible process revenue, from both ends yielding a filament known as F-actin. The filamentous actin is one of the key factor control the change between G-actin and F-actin.

The association and dissociation of monomer from both ends at high concentration of free subunits with growing barbed end is faster, however the pointed end decreases in length [10]. The monomers (G-actin) connect the fast growing barbed end (+) of the filament in the ATP state. The ATP state is more steady than ADP. The hydrolysis takes place in the filament, the ADP actin monomers dislocate faster from pointed end (-) this mechanism of actin polymerization/depolymerization is called as actin filament traedmilling. In traedmilling cycle there is net gain of monomers at the barbed end and net loss of monomers at the pointed end. The ATP binding and hydrolysis play a crucial role in the actin filament. The parallel actin filament, bundles that form the core of each stereocilium is continually revive, with entire actin bundle regularly gather at the tip (FTS), treadmilling downward and disperse at the base as depicted in figure 1 (A) . Actin binding sites effect the cross linking structure and the proteins are larger in size having spacer peptides while protein which involve in crossing actin filament into bundles, are small in size having close binding sites.

The cross linking of actin filaments, forming of parallel actin bundle is moderated by actin bundling proteins [11]. These actin bundling protein are fimbrin or plastin, villin and espin. The fimbrin is a cytoskeletal protein associated with actin bundling microfilaments of stereocilia, along with cell-substratum attachment sites [13, 14]. Fimbrin has 67kDa monomeric actin bundling protein, has two CH actin binding motifs. Fimbrin or plastin has two EF hands (calcium bind domains), however has no calcium sensitivity found, present in the hair cell stereocilia [15]. Fimbrin binds to the actin filaments as monomer, and hold two parallel filaments close together depicted in the figure 1 (B,C,D) [16].

Electron microscopy studies by Bretscher A *et.al*, suggested that fimbrin cross links to F-actin gives straight bundles, with shorter bundles of actin filament forming at high fimbrin to actin ratios. This gives information that cross linking of both in such a way to confer the rigidity of bundles formed at stereocilia. This function of fimbrin is compatible with its in-vivo localization in straight, highly organized microfilament bundles such as microvilli and stereocilia [17].

At the same time, villin is another actin bundling protein, lacking in stereocilia, found in the brush border cells. Fimbrin replaces villin in stereocilia, as the villin has a cleaving activity which is activated by calcium in contrast to this fimbrin, does not. When the stereocilia are getting activated, the calcium increases to conquer collapse of stereociliary structural integrity.

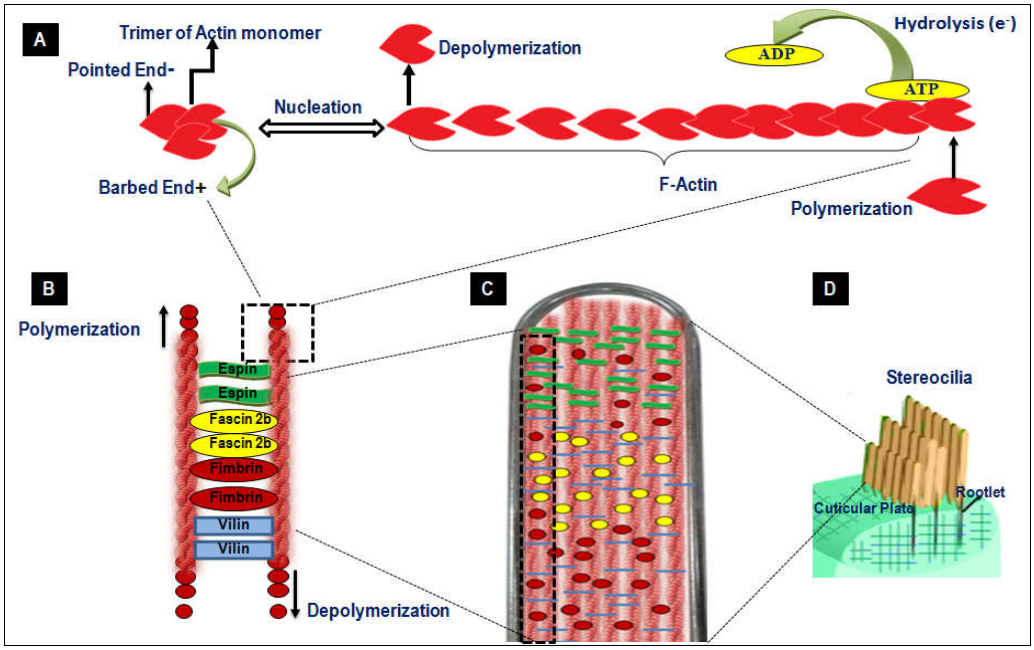

Figure 1: Mechanism of Actin filament formation:

A) Actin has minus end and plus end. The polymerization is high at plus end, simultaneously adding of G-actin to the filamentous actin (F-actin) and removal of Actin from minus end as Treadmilling.

B) Polymerization and crosslinking along with actin bundling protein espin, villin, fimbrin and Fascin 2b with disassembly and depolymerization of actin filament.

C) Sterocilium shows cross linking of actin bundling proteins.

D) Stereocilia along with cuticular plate and rootlets present in the inner ear cochlea.

One of these 55-kDa globular protein is fascin, a actin bundling protein, serves to collect F-actin into parallel bundles. These bundles are involved in cell protrusions, rearranging the cytoskeleton and promoting cellular mobilization [19]. Immunolabeling studies of Shih-Wei Chou *et al* reported that zebra fish hair cell signify that fascin 2b localized to stereocilia specifically. The study shows that fascin 2b-actin filament complexes formed parallel actin bundling in vitro, their finding denote that fascin 2b plays a crucial role in shaping stereocilia [20].

From last several years, the characterization of a new family of actin bundling proteins called as espin [21. Espin, a multifunctional actin binding and bundling protein of IHCs and OHCs of stereocilia has two isoforms. One of the isoforms has been discovered ˜110-kDa espin of sertoli cell present in spermatid junctions (ectoplasmic specialization). The other small espin isoform is having 30 kDa molecular weight, that shares the C-terminal actin bundling module, which is 116-amino acid but not N-terminal. The espin protein involves in the lengthening of stereocilia filled actin filament. Espin performs an important role in regulating the organization, dimension and signalling capacities of the stereocilia filled actin filament in mechanoreceptors and chemoreceptor cells [22]. They are the influential actin bundling protein that are not inhibited by ca2+ [21]. Espin localization to parallel actin filaments of hair cell stereocilia, efficiently elongate the parallel actin bundles, helping in length determination of stereocilia. Having modular organization and at least two or more actin binding sites per monomer (G-actin). Similarly as seen in actin filament polymerization where the cross linking of espin originate high elongation at barbed end and also slows the actin depolymerization in vitro as depicted in the figure 1(A) [25].

Espin 854 amino acid protein encoded by gene [ESPN] 1p36.1. The human Espin sequence have 8 ankyrin repeats at N-terminal, 2 proline rich regions the actin binding WH2 motif having consensus site for ATP or GTP binding and C-terminal actin bundling module [24]. The nearer module contain two f-actin binding sites predicted to form a coiled coil structure, necessary and adequate for actin bundling activity [25. Espin bind the actin monomer via their WH2 domain and assemble actin bundles in cells. The Deletion mutagenesis investigation suggested by S Naz, that espin contains 3 actin binding sites, 2 sites are on C-terminus the actin bundling module and essential for espin activity [24] and found an additional one site at N-terminal of espin [26].

Table 1: Actin bundling proteins in different models

	First author	Title of Paper	Tested model	Study design	Conclusion	References
1	Bartles *et al,* 1996	Identification and characterization of espin, an actin-binding protein localized to the F-actin rich junctional plaques of sertoli cell ectoplasmic specialization	Rat	Espin is involved in linking actin filament to each other or membrane ectoplasmic specialization of membrane cytoskeletal assembly were studied using monoclonal immunogold electron microscopy and by also espin is predicated by cDNA sequence.	Espin is involved in linking actin filaments to each other or to membranes, thereby potentially playing a key role in the organization and function of the ectoplasmic specialization.	[12]
2	Bartles *et al*, 1998	Small espin: A third actin-bundling protein and potential forked protein ortholog in brush border microvilli	Rat	Homogenates (˜4% wt/ol) prepared from the testis, kidney and small intestine mucosal scraping of adult rats in 0.25 M sucrose 3Mm imidazole, containing protease inhibitors and seperate by centrifugation PAGE and Western blotting were prepared from these various fractions by adding SDS gel. Labelled protein was determined by scanning laser densitometric analysis of Western blot.	Study strongly supported the existence of the small espin a newly identified actin bundling protein of brush border, suggested that espin binds and stabilize the f-actin.	[13]
3	S Naz *et al*, 2004	Mutations of ESPN cause autosomal recessive deafness and vestibular dysfunction.	Jerker mouse	Mutation in ESPN gene, DFNB36 responsible for recessively inherited deafness and vestibular areflexia were studied by frameshift mutations in ESPN gene which encodes a calcium- actin-bundling protein.	Espin as an essential protein for hearing and vestibular function in humans. The abnormal vestibular phenotype associated with ESPN mutations will be a useful clinical marker for refining the differential diagnosis of nonsyndromic deafness.	[25]
4	Redouance *et al*, 2008	A novel mutation in the espin gene causes autosomal recessive nonsyndromic hearing loss but no apparent vestibular dysfunction in a Moroccan family	ARNSHIL family	Identification of novel DFNB36 loci was facilitated by analysis of Autosomal recessive nonsydromic hearing loss (ARNSHL) and also studied recessive ESPN mutation causing congenital hearing loss.	The studied conclude that novel c.1757insG mutation in ESPN gene provide further evidence that truncating C-terminal mutation represent common in recessive hearing loss.	[26]
5	G Sekerkova, 2011	Roles of espin actin bundling proteins in the morphogenesis and stabilization of hair cell stereocilia	Jerker mouse strain CBA/CaJ strain	The congenic jerker mouse line backcrossing into the inbred CBA/CaJ strain and compared stereocilia in wild-type	Espin actin bundling proteins are required for the assembly and stabilization of the stereociliary parallel actin bundle.	[28]

		revealed in CBA/CaJ congenic jerker mouse		CBA/CaJ mice, jerker homozygotes owing to a frameshift mutation in the espin gene.		
6	Rzadzinska B *et al,* 2005	Balanced levels of espin are critical for stereociliary growth and length maintenance	Jerker mouse	Actin-bundling protein Espin in stereociliary growth were studied by examining the hair cell stereocilia of Espin-deficient jerker mice (Espn(je)) and the effects of transiently over expressing Espin in the neuroepithelial cells of the organ of Corti cultures using fluorescence scanning confocal and electron microscopy.	Studied concluded that Espin is important for the growth and maintenance of the actin-based protrusions of inner ear neuroepithelial cells.	[29]

CONCLUSION

Noise induced hearing loss is disease caused due to loss of panel of proteins working together for hearing acuity. This review focused on the actin bundling proteins fimbrin, villin, espin and fascin work in coexistence with different combinations for formation of filament cross-linking in cytoskeleton of actin filled stereocilial structure. The congruity of these proteins comes to form parallel actin bundles for highly specific functions, exhibits different binding affinities and modes of regulation to assemble these fascinating combinations on the bundling theme. Actin bundle formation facilitates the proper assembly and the cellular specialization with which it is associated. However suggest that each actin bundling protein is essential and play a specific key role, while mutation or eliminations in the actin bundling protein has variable effect on the parallel actin bundles. During diseased condition, different actin-bundling proteins might hinder in cross linking of actin bundle, which is still a great deal to learn utilization of a particular set of actin-bundling proteins and has bigger area open for research.

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REFERENCES

1. Pingle, S., Tumane, R., Jawade, A., Jain, R., Thakkar, L., Sishodiya, P. & Mandal, B. (2013). Evaluation of biomarkers for the early detection of noise induced hearing loss in the mine workers. Project Report Vol., (1)3:123.
2. Noise Induced Hearing Loss clinical preservation, Available from: https://www. emedicine. medscape.com
3. Noise-Induced-Hearing-Loss, Available from: https://www.nidcd.nih.gov/health/
4. Ear Anatomy, Available from: https://www.myvmc.com/anatomy/ear/
5. Stereocilia, Available from: http://en.m.wikipedia.org/Steriocilia
6. Loomis, P.A., Zheng, L., Sekerková, G., Changyaleket, B., Mugnaini, E. & Bartles, J.R. (2003). Espin cross-links cause the elongation of microvillus-type parallel actin bundles in vivo. J. Cell Biol., 163(5):1045-55.
7. Komaba, S., Inoue, A., Maruta, S., Hosoya, H. & Ikebe M. (2003). Determination of human myosin III as a motor protein having a protein kinase activity. J. Biol. Chem., 278(24):21352-60.
8. Salles, F.T., Merritt, R.C., Jr, Manor, U., Dougherty, G.W., Sousa, A.D., Moore, J.E., Yengo, C.M., Dosé, A.C. & Kachar, B. (2009). Myosin IIIa boosts elongation of stereocilia by transporting espin 1 to the plus ends of actin filaments. Nat. Cell Biol., 11(4):443-50.
9. Cooper, G.M. & Sunderland, M.A. (2000). The Cell: A Molecular Approach. 2nd edition. Sinauer Associates.
10. Dominguez, R. & Holmes, K.C. (2011). Actin structure and function. Annu Rev Biophys., 40:169-86.
11. Actin filaments, Available from: https://www.youtube.com/watch?v=-EEwp-H0ENw
12. Bartles, J.R., Wierda, A. & Zheng, L. (1996). Identification and characterization of espin, an actin-binding protein localized to the F-actin-rich junctional plaques of Sertoli cell ectoplasmic specializations. J. Cell Sci., 109 (Pt 6):1229-39.

13. Bartles, J.R., Zheng, L., Li, A., Wierda, A. & Chen, B. (1998). Small espin: a third actin-bundling protein and potential forked protein ortholog in brush border microvilli. J Cell Biol., 143(1):107-19.
14. Bretscher, A. (1981). Fimbrin is a cytoskeletal protein that crosslinks F-actin invitro. Proc. Natl. Acad. Sci. USA, N78(11):6849-53.
15. Lakowski, T.M., Lee, G.M., Okon, M., Reid, R.E. & McIntosh, L.P. (2007). Calcium-induced folding of a fragment of calmodulin composed of EF-hands 2 and 3. Protein Sci., 1119-32.
16. Daudet, N. & Lebart, M.C. (2002). Transient expression of the t-isoform of plastins/fimbrin in the stereocilia of developing auditory hair cells. Cell Motil. Cytoskeleton, 53(4):326-36.
17. Flock, A., Bretscher, A. & Weber, K. (1982). Immunohistochemical localization of several cytoskeletal proteins in inner ear sensory and supporting cells. Hear Res., (1):75-89.
18. Bretscher, A. (1981). Fimbrin is a cytoskeletal protein that crosslinks F-actin invitro. Proc. Natl. Acad. Sci USA, 78(11):6849-53.
19. George, S.P. & Wang, Y., Mathew, S., Srinivasan, K. & Khurana S. (2007). Dimerization and actin-bundling properties of villin and its role in the assembly of epithelial cell brush borders. J. Biol. Chem., 282(36):26528-41.
20. Roma, A.A. & Prayson, R.A. (2005). Fascin expression in 90 patients with glioblastoma multiforme. Ann. Diagn. Pathol., (6):307-11.
21. Chou, S.W., Hwang, P., Gomez, G., Fernando, C.A., West, M.C., Pollock, L.M., Lin-Jones, J., Burnside, B. & McDermott, B.M. Jr. (2011). Fascin 2b is a component of stereocilia that lengthens actin-based protrusions. PLOS. One., 6(4):e14807.
22. Zheng, L., Sekerková, G., Vranich, K., Tilney, L.G., Mugnaini, E. & Bartles, J.R. (2000). The deaf jerker mouse has a mutation in the gene encoding the espin actin-bundling proteins of hair cell stereocilia and lacks espins. Cell., 102(3):377-85.
23. Espin protein Available from: https://www.uniprot.org/uniprot/B1AK53
24. Sekerková, G., Zheng, L., Loomis, P.A., Mugnaini, E. & Bartles, J.R. (2006). Espins and the actin cytoskeleton of hair cell stereocilia and sensory cell microvilli. Cell Mol. Life Sci., 63(19-20):2329-41.
25. Naz, S., Griffith, A.J., Riazuddin, S., Hampton, L.L., Battey, J.F. Jr., Khan, S.N., Riazuddin, S., Wilcox, E.R. & Friedman, T.B. (2004). Mutations of ESPN cause autosomal recessive deafness and vestibular dysfunction. J. Med. Genet., 41(8):591-5.
26. Boulouiz, R., Li, Y., Soualhine, H., Abidi, O., Chafik, A., Nürnberg, G., Becker, C., Nürnberg, P., Kubisch, C., Wollnik, B. & Barakat A. (2008). A novel mutation in the Espin gene causes autosomal recessive nonsyndromic hearing loss but no apparent vestibular dysfunction in a Moroccan family. Am. J. Med. Genet. A., 146A(23):3086-9.
27. Chen B, Li A, Wang D, Wang M, Zheng L, Bartles JR. Espin contains an additional actin-binding site in its N terminus and is a major actin-bundling protein of the Sertoli cell-spermatid ectoplasmic specialization junctional plaque. Mol Biol Cell. 1999 Dec;10(12):4327-39. PubMed PMID: 10588661; PubMed Central PMCID: PMC25761.
28. Sekerková, G., Richter, C.P. & Bartles, J.R. (2011). Roles of the espin actin-bundling proteins in the morphogenesis and stabilization of hair cell stereocilia revealed in CBA/CaJ congenic jerker mice. PLoS. Genet., 7(3):e1002032.
29. Rzadzinska, A., Schneider, M., Noben-Trauth, K., Bartles, J.R. & Kachar B. (2005). Balanced levels of Espin are critical for stereociliary growth and length maintenance. Cell Motil. Cytoskeleton, 62(3):157-65.