



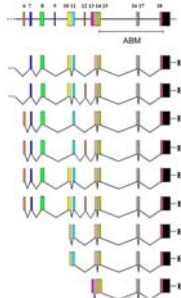
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THE ESPIN FAMILY OF ACTIN BUNDLING PROTEINS

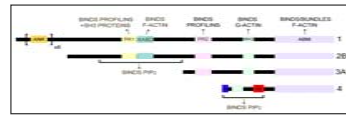
GENERAL RESEARCH INTERESTS

- * The processes by which regulatory sequences within the Espin pre-mRNA and the proteins which bind them act in concert to modulate splice site selection
- * Determination of how the Espin promoters can purposely recruit RNA processing and regulatory factors that function to delineate the splicing of nascent transcripts into mature mRNA
- * Characterization of Zebrafish (*Danio rerio*) Espins and the role they play in the development and maintenance of the auditory and vestibular systems of the inner ear

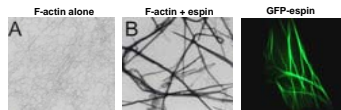
BACKGROUND



Espins are encoded by a single gene. The isoforms are delineated by differential start site selection and alternative splicing.



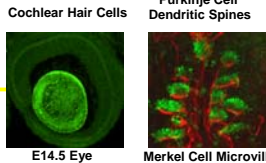
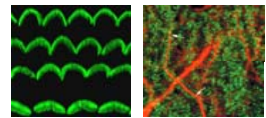
Examples of the four classes of Espins highlighting domains and activities. ANK: ankyrin-like repeat; PR: proline rich domain; XABS: additional F-actin binding site; WH2: WASP homology 2 domain; ABM: actin bundling module.



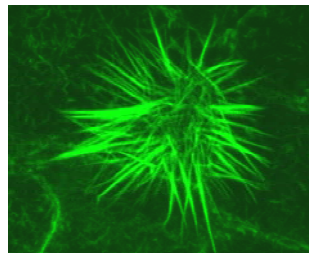
Espins bind and bundle actin with a $K_D \sim 70$ nM



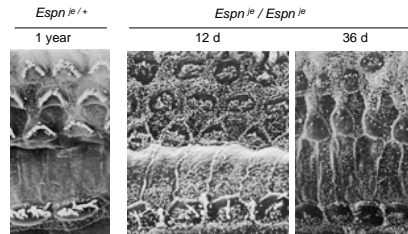
Sequence comparison between mouse, human and zebrafish Espin 1. Sequence conservation with zebrafish Espin 1 is indicated by yellow shading. * Ankyrin-like repeats domain; * Proline rich domain; * WH2 domain; Actin binding domain.



Espins (green) are found in a variety of rodent chemosensory and mechanosensory cells.



Espin cross-links result in the dramatic elongation of microvillus type parallel actin bundles within transfected CL4 cells



The absence in mice of Espins due to mutation (Jerker, *je*) results in the loss of stereocilia and hair cell degeneration. Human Espin mutations resulting in deafness and vestibular dysfunction have also been identified (J Med Genet (2004) 41:591; (2005) 43:157).

PROJECT 1: REGULATION OF THE ALTERNATIVE SPLICING OF HUMAN AND MOUSE ESPIN PRE-mRNA

MAIN RESEARCH TECHNIQUES:

1. Site directed mutagenesis
2. PCR
3. DNA/RNA binding assays
4. Transfection of cultured cells
5. Knock-down assays of RNA binding proteins
6. Luciferase-directed assays to determine promoter activity
7. Western blotting

PROJECT 2: CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF ESPINS WITHIN ZEBRAFISH INNER EAR

MAIN RESEARCH TECHNIQUES:

1. RACE and Nested PCR
2. Cloning
3. Gene knock-out
4. Luciferase-directed assays to determine promoter activity
5. Northern blotting
6. Western blotting
7. Actin binding and bundling assays
8. Protein purification – tissue and bacterial expressed proteins
9. Yeast-two hybrid analysis
10. Indirect immunofluorescence microscopy
11. Morpholino injection of zebrafish eggs
12. *In situ* hybridization
13. Electron microscopy

ESPIN PUBLICATIONS

- Sekerková Sekerkova, G; Loomis, PA; Changyaleket, B; Zheng, L; Eytan, R; Chen, B; Mugnaini, E; Bartles, JR. (2003) Novel espin actin-binding proteins are localized to Purkinje cell dendritic spines and bind the Src homology 3 adapter protein insulin receptor substrate p53. *J. Neurosci.* 23(4):1310-1319.
- Loomis, PA; Zheng, L; Sekerková Sekerkova, G; Changyaleket, B; Mugnaini, E; Bartles, JR. (2003) Espin cross-links cause the elongation of microvillus-type parallel actin bundles in vivo. *J. Cell Biol.* 163(5):1045-1055.
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- Loomis, P.A., Kelly, A.E., Zheng, L., Changyaleket, B., Sekerková Sekerkova, G., Mugnaini, E., Ferreira, A., Mullins, R.D., Bartles, J.R. (2006) Targeted Wild-Type and Jerker Espins Reveal A Novel WH2 Domain-Dependent Way To Make Actin Bundles in Cells. *JCS.* 119(8):1655-1665.
- Sekerková G, Zheng L, Loomis PA, Mugnaini E, Bartles JR. Espins and the actin cytoskeleton of hair cell stereocilia and sensory cell microvilli. (2006) *Cellular and Molecular Life Sciences.* 63:2329-2341.

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