

Javelin, a novel protein is essential in Drosophila bristle actin bundles formation

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In several specialized cell types, such as intestinal microvilli, stereocilia of the inner ear and *Drosophila* bristles, the structural integrity requires a specific actin filament organization brought about by a set of actin bundling proteins. In *Drosophila* bristle development, two major actin bundling proteins – Forked and Fascin are involved in forming actin networks but only little is known about the utilization of these proteins in a time- and space- dependent manner. In our present study, using RNA sequence analysis a new gene, Javelin (*Jv*), was identified that directly affect actin bundles formation. Javelin mutant bristles do not taper like bristles in wild-type flies, instead they present a small enlargement before the tip. *In-vitro* and *in-vivo* studies using Javelin in the presence of Forked and Fascin showed that *jv* plays a role in actin bundling in *Drosophila*.

Javelin (jv) mutants show aberrant bristle morphology







Bristle tip becomes swollen, resembling a spear



Irregular groove pattern with unparallel and shallower ridges

Purification of Javelin (Jv) and Co-immunoprecipitation assay $\longrightarrow Jv 170 \text{ kDa}$ ABCWestern blot showing Co-IP ofElag - layelin and GEP Forked by

GFP ForkedFlag Javelin and GFP Forked by
anti-Flag and anti-GFP antibody.GFP ForkedCo-immunoprecipitation (Co-IP)
shows no interaction between
Flag Javelin and GFP Forked in
Drosophila ovary lysate.

(A) represents the purified flag-

javelin protein, (B) represents the



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Gel image showing the purified Javelin (MW 170 kDa) from SF-9 cells

GFP Forked ovary lysate and (C) represents the GFP beads bound with both Flag Javelin and GFP Forked.

Javelin was cloned into two parts – N terminus and C-terminus along with the Coiled Coil Domain (CCD) and was fused with EGFP



Schematic representation of the domain structures of Javelin full length, Javelin N-terminus and Javelin C-terminus respectively.

Jv consists of only one functional domain – CCD, which has no actin bundling activity. Inspite of this, Jv has a role in actin network thus making it an interesting candidate.

Jv full length, Javelin N-terminus and C-terminus exhibit unique localization pattern in Drosophila oocyte



GFP Javelin N-Terminus/Mat Gal 4-3



Confocal projections showing the ectopic expression of GFP Forked, Fascin and Javelin and their different combinations in *Drosophila* oocyte and the resulting actin networks.

100 µm

When expressed with Forked, *Jv* N-terminus inhibits actin bundling whereas *Jv* C-terminus favours actin bundling





Confocal projections showing the localization pattern of Javelin full length (A), Nterminus of javelin (B) and C-terminus of Javelin (C) when driven with a maternal Gal4-3.



Confocal projections showing the effect of Javelin full length (A-C), Javelin Nterminus (D-E) and Javelin C-terminus (G-H) on the actin bundles when expressed with mCherry Forked.