Slit-Robo signalling establishes a Sphingosine-1-phosphate gradient to polarise fin mesenchyme

Ng Ee Chern, Ong Yi Xuan, Rebecca, and Tay Jun Yu

Lee Kong Chian School of Medicine, Nanyang Technological University-Imperial College London, Singapore

INTRODUCTION

- Interaction between mesenchymal cells and apical ectodermal ridge (AER) promote migration of cells into the growing fin[1]
- Subsequent tensile forces between these cells drive morphogenesis[2]
- Sphingosine-1-phosphate (S1P) promotes cell migration, adhesiveness and myosin-based contractile tension in mesenchymal cells and fibroblasts[3]
- BUT pathways defining the regulation of intra and extracellular S1P levels are not fully known

OBJECTIVES

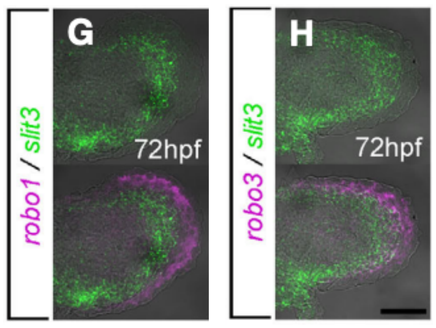
- Understand the processes driving mesodermal cell polarisation, migration and organisation in the limb and the biophysical properties they impart
- Show that Slit-Robo signalling is required for S1P potency in the fin fold
- Show that Slit-Robo and S1P coordinate to provide tension to the interstitial matrix of the fin, driving robust tissue morphogenesis

METHODS

(1) Genetic mapping of stomp to Slit3 → (2) Establish mechanism behind Slit3's role in integrity of fin morphogenesis → (3) Determine the presence of synergy between Slit-Robo and S1P signalling pathways and their sequential order → (4) Establish importance of S1P in fin elongation and polarity and its mechanism → (5) Explain how a self-generated signalling gradient directs mesenchyme migration in fin morphogenesis

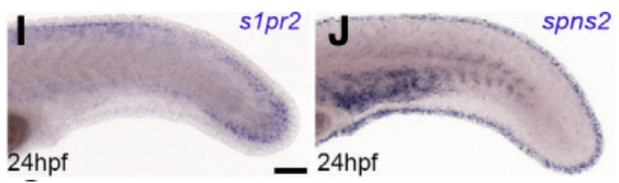
RESULTS

1) Slit3 ligands derived from immigrating mesenchymal cells signal through Robo receptors at the apical epidermal ridge (AER) to influence fin morphogenesis.



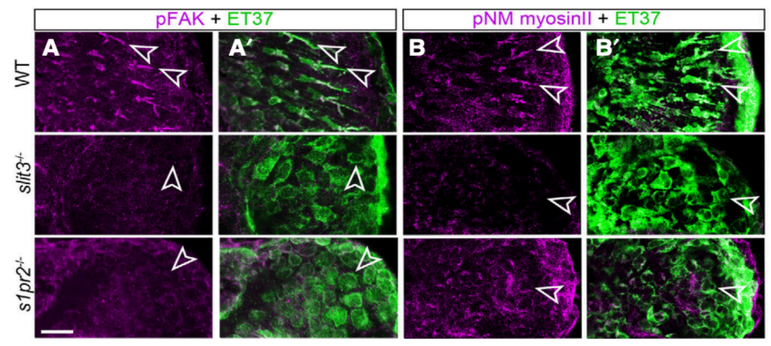
In-situ hybridisation of the zebrafish fins showing slit3 (green) binding to either robo1 or robo3 (magenta)

(2) The Slit-Robo signalling pathway promotes the release of S1P from the AER, which in turn acts in the S1P receptor 2 (s1pr2) on mesenchymal cells.

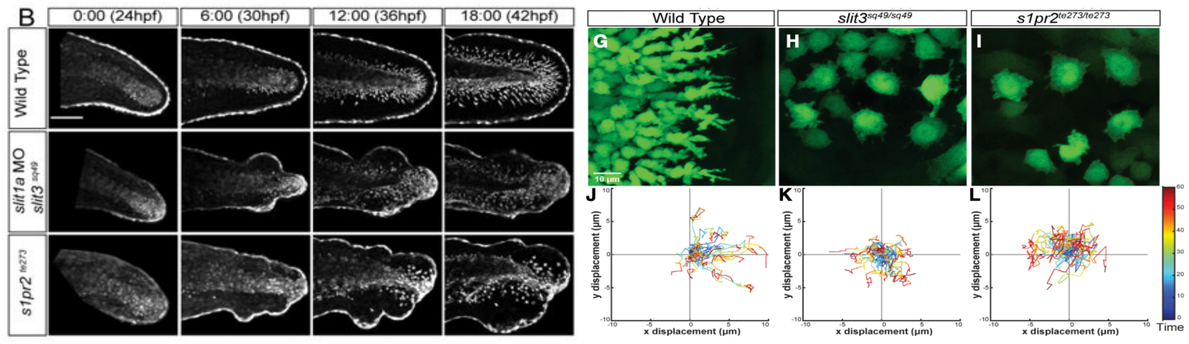


In-situ hybridisation of zebrafish fins showing s1pr2 expression in the migrating mesenchyme (image I) and expression of spns2, the S1P exporter, at the AER (image J)

(3) Mutations to either the Slit3 gene or the s1pr2 gene results in defects in mesenchyme behaviour, loss of polarisation and adhesion. This leads to defective fin morphogenesis and blister formation.

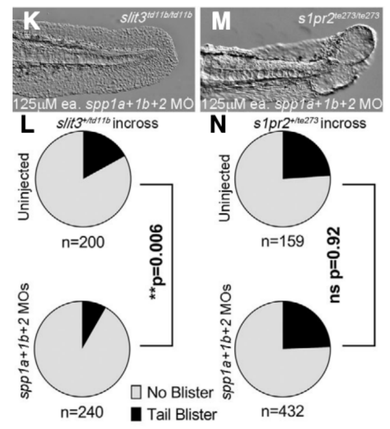


Immunofluorescent staining of zebrafish fins showing significantly reduced focal adhesion kinase (FAK) and myosin II in slit3 mutants and s1pr2 mutants compared to wildtype (WT) fin mesenchyme (images A-B). Unlike wildtypes, mutant mesenchymal cells fail to migrate into the fin normally, resulting in blisters (image B).



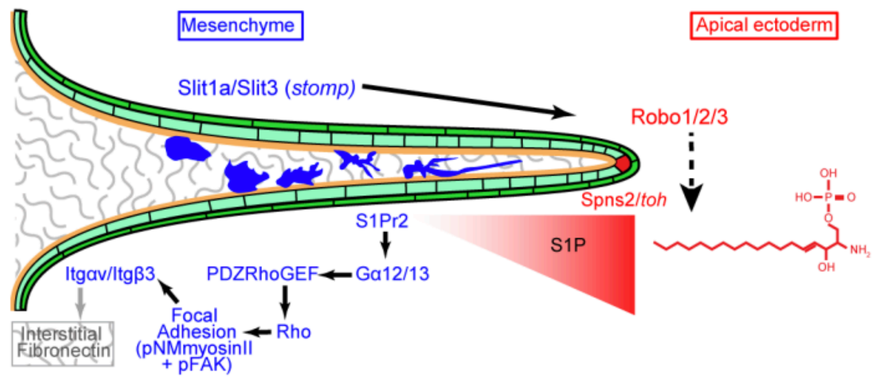
There is also a loss of elongation and polarisation (images G-I) as well as lack of displacement (images J-L) in both slit3 and s1pr2 mutants.

(4) Reducing the dephosphorylation of S1P by introducing S1P phosphatases resulted in a lowering of the frequency of blistering in slit3 mutants but not s1pr2 mutants, showing that S1P production is epistatic to Robo function, and that increasing S1P levels partially rescues the loss of Slit3 mutants.



Reduction of S1P phosphatases (spp1a and 1b) reduces the number of zebrafish larvae with blisters

(5) The binding of S1P to s1pr2 on mesenchymal cells to promotes the polarisation, orientation, positioning by inducing stress fibres and focal adhesions in interstitial matrix of the fin fold via Rho.



Slit-Robo signalling promotes the release of S1P from the AER, resulting in a gradient that acts back of mesenchymal cells which express s1p2r to regulate polarity and migration, culminating in adhesion to interstitial fibronectin

REFERENCES

1. Gros J, Hu JK-H, Vinegoni C, Feruglio PF, Weissleder R, Tabin CJ. WNT5A/JNK and FGF/MAPK pathways regulate the cellular events shaping the vertebrate limb bud. Current Biology. 2010;20(22):1993–2002.
2. Wada N. Spatiotemporal changes in cell adhesiveness during vertebrate limb morphogenesis. Developmental Dynamics. 2011;240(5):969–78.
3. Wang F, Nobes CD, Hall A, Spiegel S. Sphingosine 1-phosphate stimulates rho-mediated tyrosine phosphorylation of focal adhesion kinase and Paxillin in Swiss 3t3 fibroblasts. Biochemical Journal. 1997;324(2):481–8.

DISCUSSION AND CONCLUSION

This project has uncovered the role of the Slit-Robo pathway in the morphogenesis of zebrafish fins and its interaction with S1P released from fin AER, thus identifying a novel relay signalling system between migrating mesenchyme and fin AER. Given this, it may be worth revisiting the significance of S1P signalling in human limb development.