Yap:Ephrin Interactions in Skeletal Muscle

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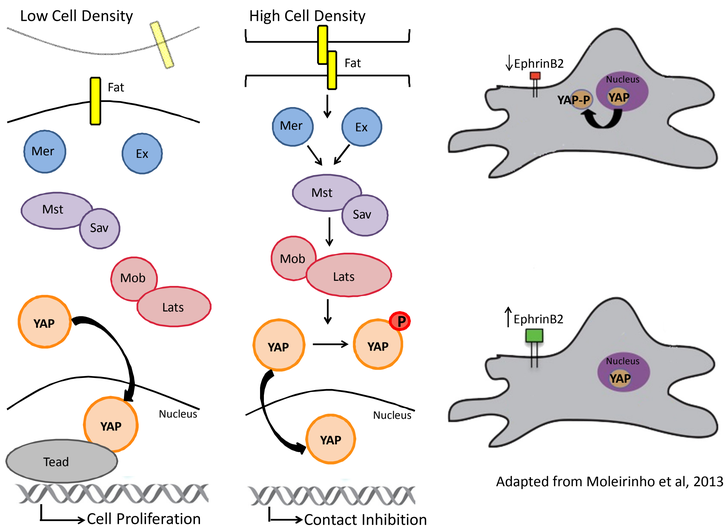
Abstract

Yes-associated protein (Yap) is a transcriptional co-factor located in the Hippo signal transduction pathway. This pathway controls organ growth but also participates in control of actin cytoskeleton polarization and cell migration. In skeletal muscle, Yap promotes satellite cell proliferation thus inhibiting differentiation. Although the signals to which Yap responds are not completely understood, it is hypothesized that Yap senses mechanical properties of the cell niche and cell to cell contact. In response to these signals Yap regulates proliferation (low when cell-cell contact is high), differentiation (only when cell-cell contact is high), migration and apoptosis. Recent work in the Camargo lab has demonstrated that ephrins are a novel mediator for the Hippo pathway. Ephrins are molecules associated with cell migration during development with different members of the ephrin family found at different stages of muscle development or health (i.e. young, old, damaged, undamaged). Previous studies in the Cornelison lab have suggested that satellite cells are influenced by ephrin signaling. We plan to overexpress constitutively active hYAP1 (S127A) versus empty vector in these cells to test whether satellite cells with Yap hyperactivity ignore ephrin boundaries and/or proliferate excessively. Moreover, because both proteins exhibit cytoskeletal effects, questions arise asking whether Hippo and Eph/ephrin signaling act in concert to organize nascent myofibers into their stereotypical parallel arrays.

Background

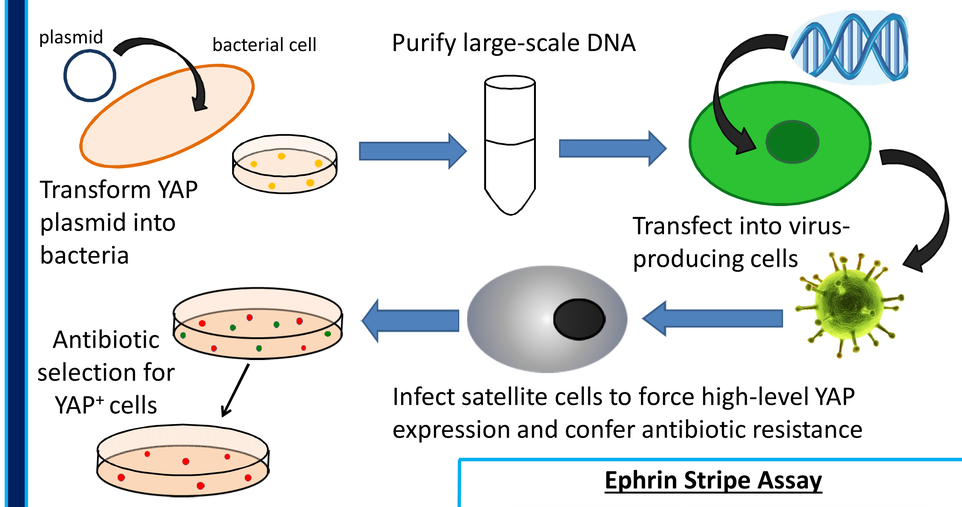


Adapted from Zhao et al, 2007

Adapted from Moleirinho et al, 2013

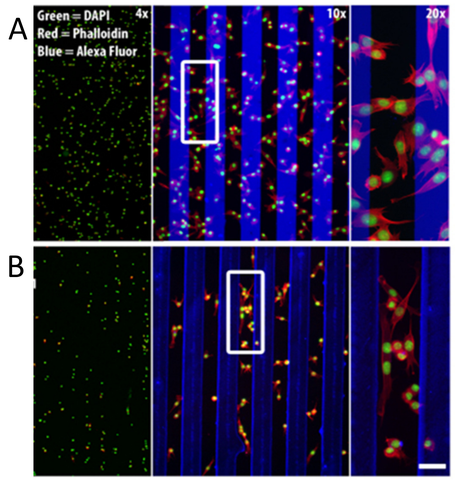
Phosphorylation of Yap occurs when the Hippo pathway is activated during high cell-cell contact. Yap phosphorylation precedes translocation into the cytoplasm where it is inactivated, and ephrins are subsequently downregulated, thus inhibiting cell proliferation. Non-phosphorylated Yap (active) remains within the nucleus where it functions as a transcriptional co-factor, promoting cell proliferation and upregulating ephrin expression.

Experimental Plan



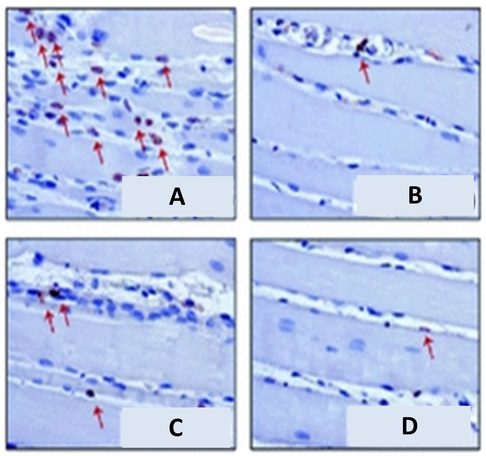
- Used to expose guidance cues and mechanisms
- Unravels potential guidance properties of extracellular matrix components
- Allows assessment of guidance activity from established molecules
- Coverslips are programmed with ephrin stripes
- Primary satellite cells are plated on prepared coverslip
- Use time lapse analysis
- We expect Yap$^+$ cells to ignore Ephrin stripe boundaries (A) and Yap$^-$ cells to remain confined (B)

Ephrin Stripe Assay



Adapted from Stark et al, 2011

BrdU Assay



Adapted from Stratos et al, 2012

- Detects 5-Bromo-2'-deoxyuridine (BrdU)
- BrdU is a pyrimidine analog, it replaces thymidine in newly synthesized DNA
- BrdU has a higher binding affinity than thymidine
- Used to distinguish proliferating cells
- Add fluorescently-labeled antiBrdU antibodies to mark proliferating cells
- We expect Yap$^+$ cells to proliferate rapidly (A, C) and Yap$^-$ cells will not proliferate (B, D)

Potential Results

- **Constitutively Active Yap**
  - Increase in ephrin expression
  - Satellite cells do not obey ephrin boundaries
  - Increase in proliferation of satellite cells
  - Increase in activation of satellite cells
  - Decrease in differentiation of satellite cells
- **Empty Vector**
  - No change in ephrin expression
  - Satellite cells obey ephrin boundaries
  - No change in satellite cell proliferation, activation, or differentiation

Conclusion:

- Effect on Regeneration:
  - In the constitutively active Yap, although the satellite cells are proliferating continuously, they will not be able to differentiate into mature myofibers. Therefore, regeneration is enhanced with the increase in satellite cells but stunted when they reach the point of differentiation
  - In the empty vector there is no effect on Yap and it would act as it does normally
- Organization of Myofibers
  - Both the Hippo pathway and the Eph/ephrin signaling pathway work together in the organization of muscle fibers
  - Eph/ephrin repulsion is important in maintaining muscle type patterning
  - Hippo pathway is required to polarize actin cytoskeleton
  - The state of Yap can increase or decrease ephrin possibly having an effect on this system

Future Studies

The roles of the Eph/ephrin pathway and the Hippo/Yap pathway in muscle regeneration are not yet identified. If it is established that the Eph/ephrin and Hippo/Yap signaling pathways interact in muscle satellite cells, future studies will aim to identify specific Eph/ephrin proteins involved in Yap signaling. Ideally, this work will continue *in vivo* by testing genetic mouse mutants for specific Ephs and ephrins as well as Hippo and Yap.

Acknowledgements

Special thanks to Danny Stark, Dane Lund, and Patrick McAnulty

Funding for this project provided by NIH grant AR062836 to DDWC and an endowment established by IDEXX-RADIL

This work will be done in collaboration with Dr. Henning Wackerhage, University of Aberdeen, who conceived the project and provided the YAP plasmids.