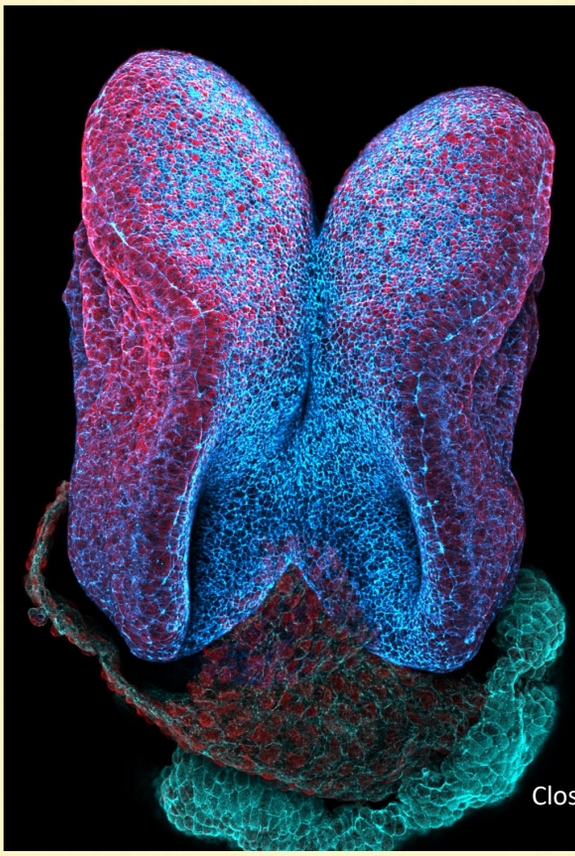


Closing mouse cranial neural tube

Apical Constriction

Gabriel Galea

UCL GOS Institute of Child Health

2023

Objectives

- **Describe apical constriction as a force-generating epithelial cell behaviour which changes tissue shape**
- **Understand key molecular regulators and effectors of apical constriction**
- **Appreciate differences in initiation and execution of apical constriction between epithelia**
- **Discuss failure of apical constriction as a cause of congenital malformations**

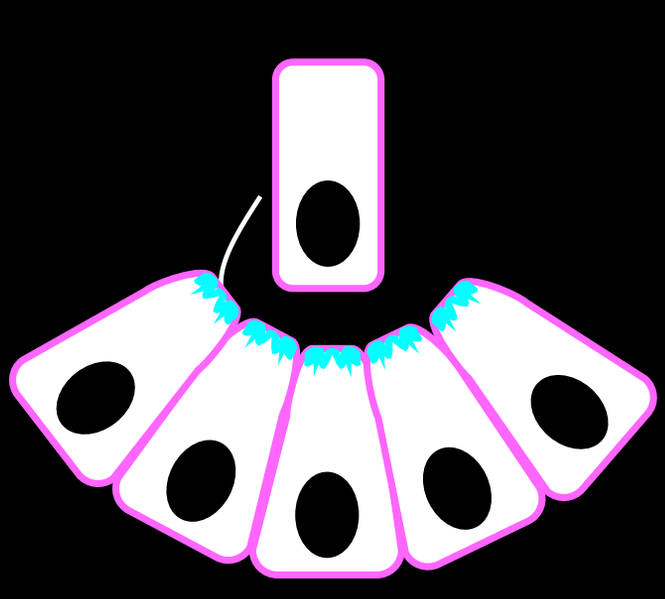
What is an epithelium?

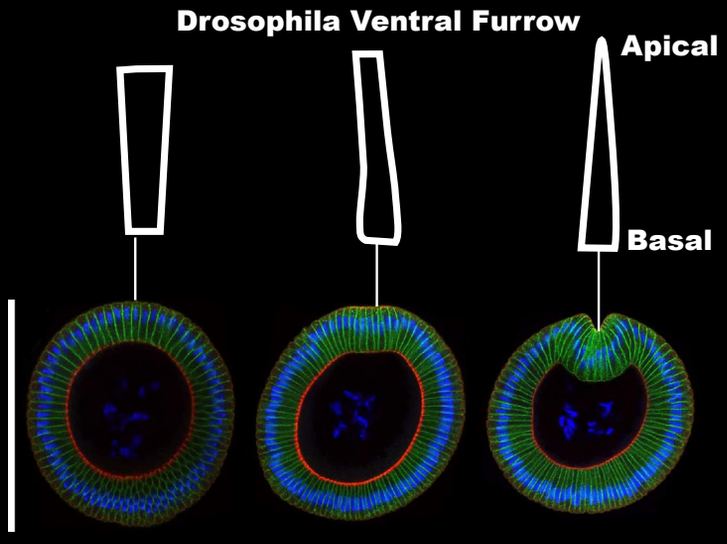
How are epithelial cells different from mesenchymal cells?

Cell-cell junctions. in epithelial

Cell-ECM in mesenchymal.

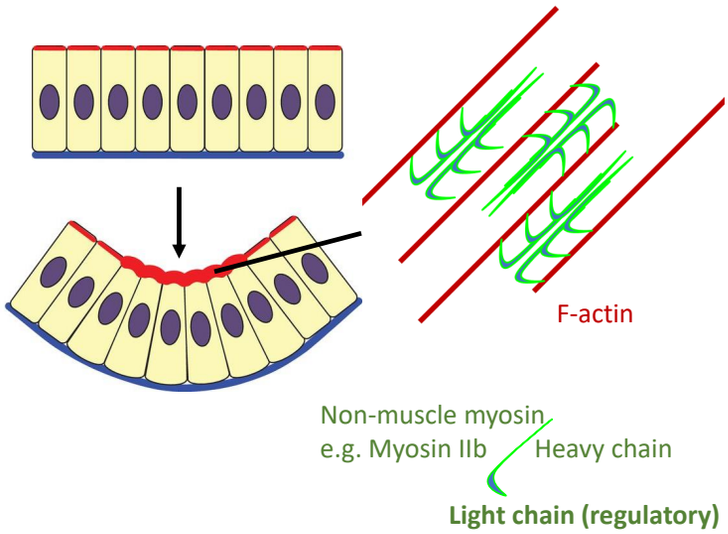
Apical constriction is a common mechanism by which epithelial cells bend their tissue





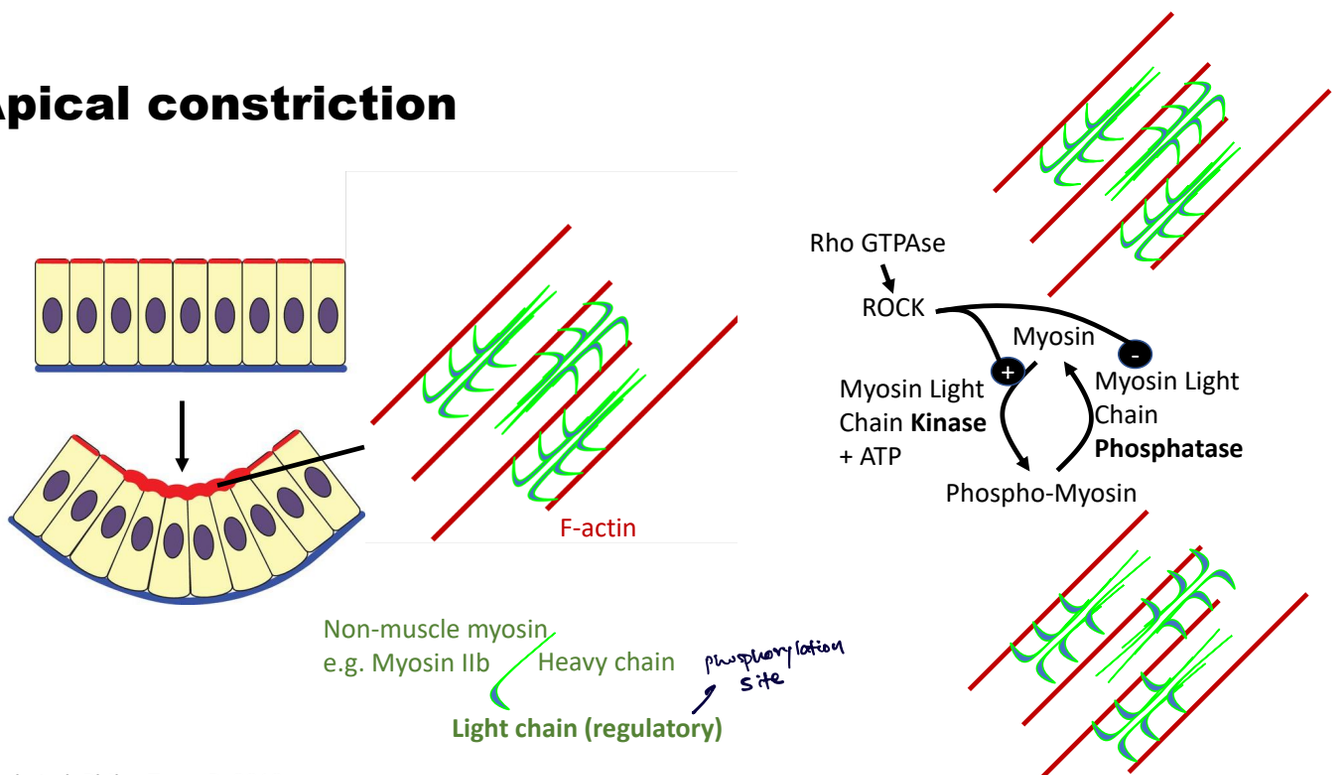
Holcomb et al Plos Comp Biol 2021

Apical constriction



Pearl et al, Philos Trans B, 2018

Apical constriction



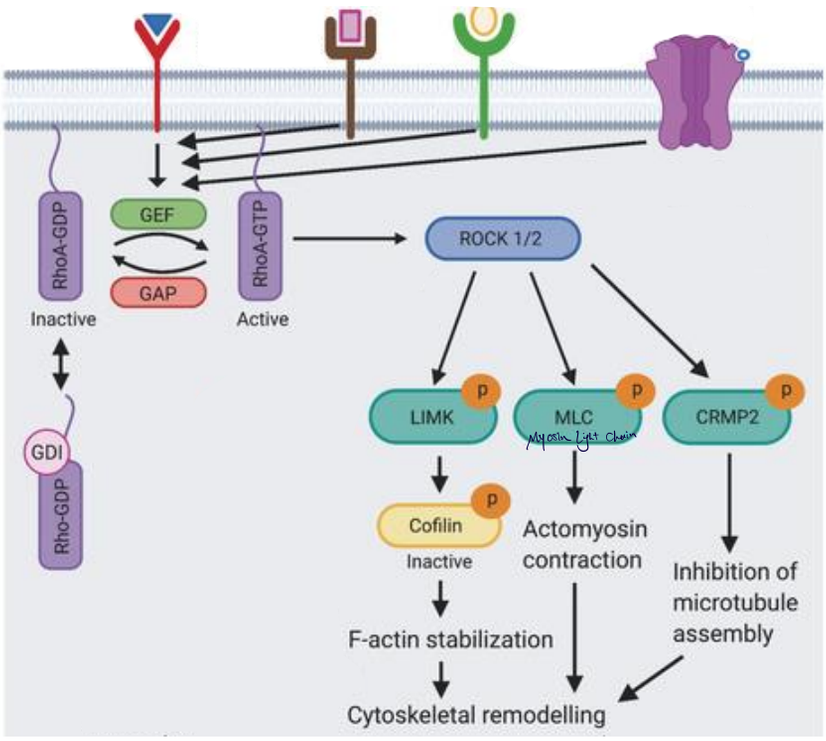
Pearl et al, Philos Trans B, 2018

Myosin in apical constriction ≠ muscle myosin.

↑
Myosin II

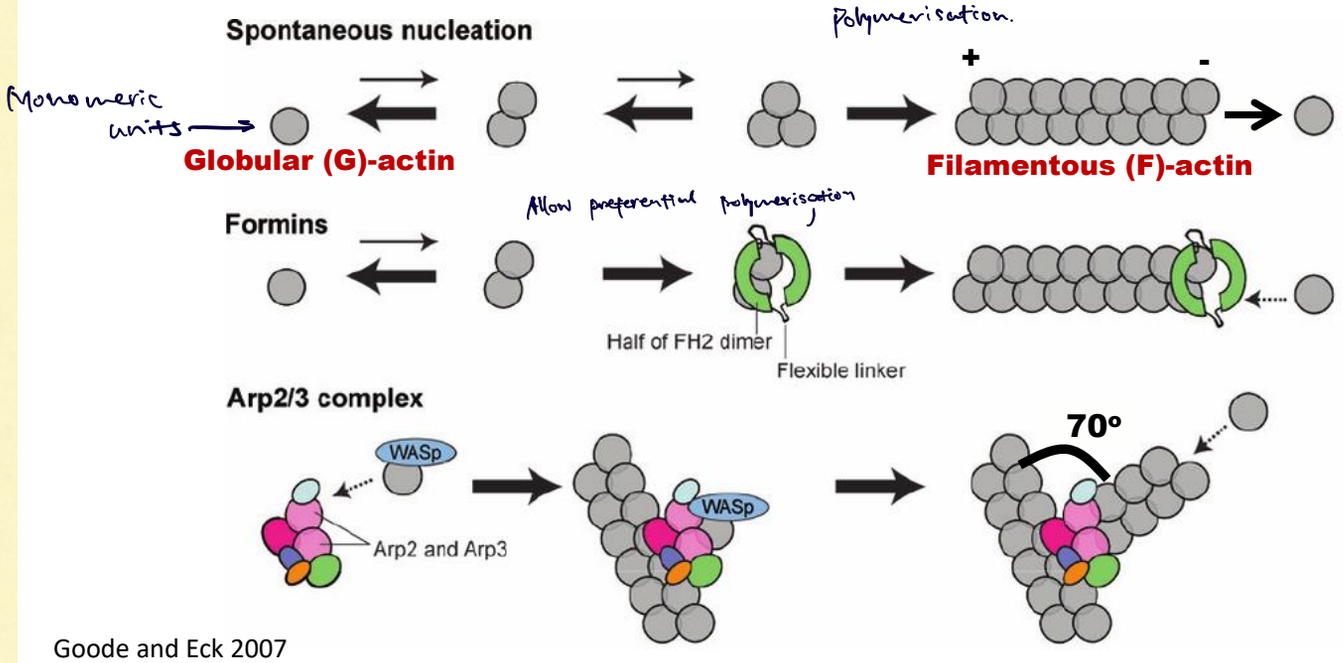
The Rho-ROCK-Myosin pathway

GTP/GDT = Guanosine tri/di-phosphate
GEF = Guanine exchange factor
GAP = GTPase activating protein
ROCK = Rho-associated kinase
LIMK = Lim kinase
MLC = Myosin light chain



Mulherkar and Tolias 2020

Myosin needs an F-actin scaffold to pull on



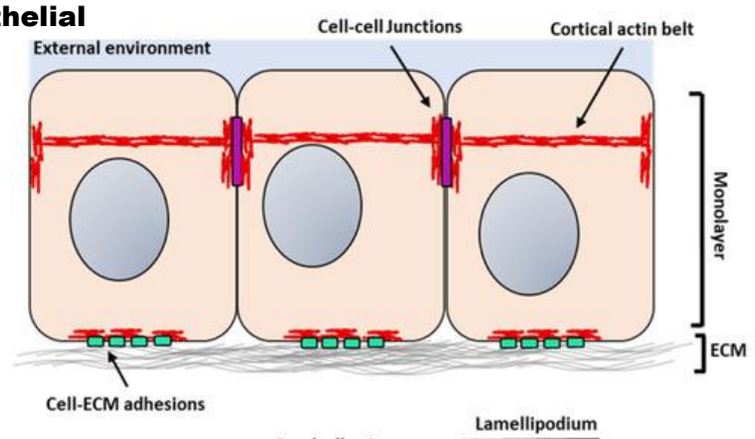
Goode and Eck 2007

In epithelial cells organisation around apical end.

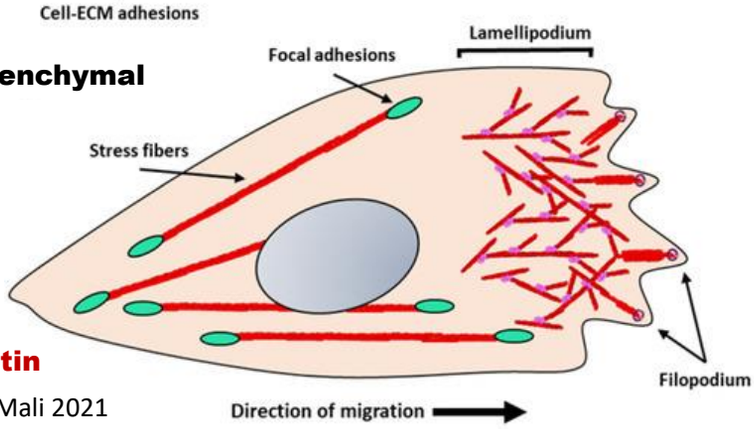
Cell-Cell junctions physically link the cytoskeleton between epithelial cells

Cortex = thick ring of F-actin and myosin around the cell's apical surface

Epithelial



Mesenchymal

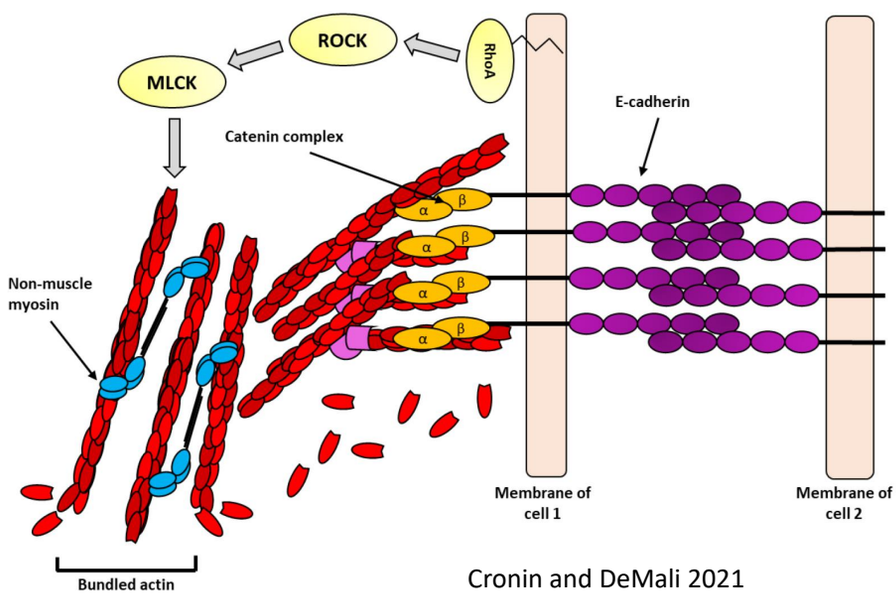


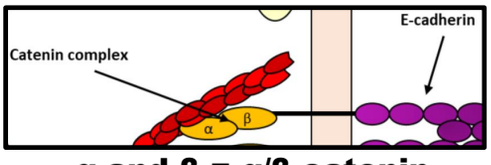
F-actin

Cronin and DeMali 2021

Cadherin/catenin Adherens Junctions are the main force-transmitting junctions in vertebrates

β-catenin used in Wnt pathway

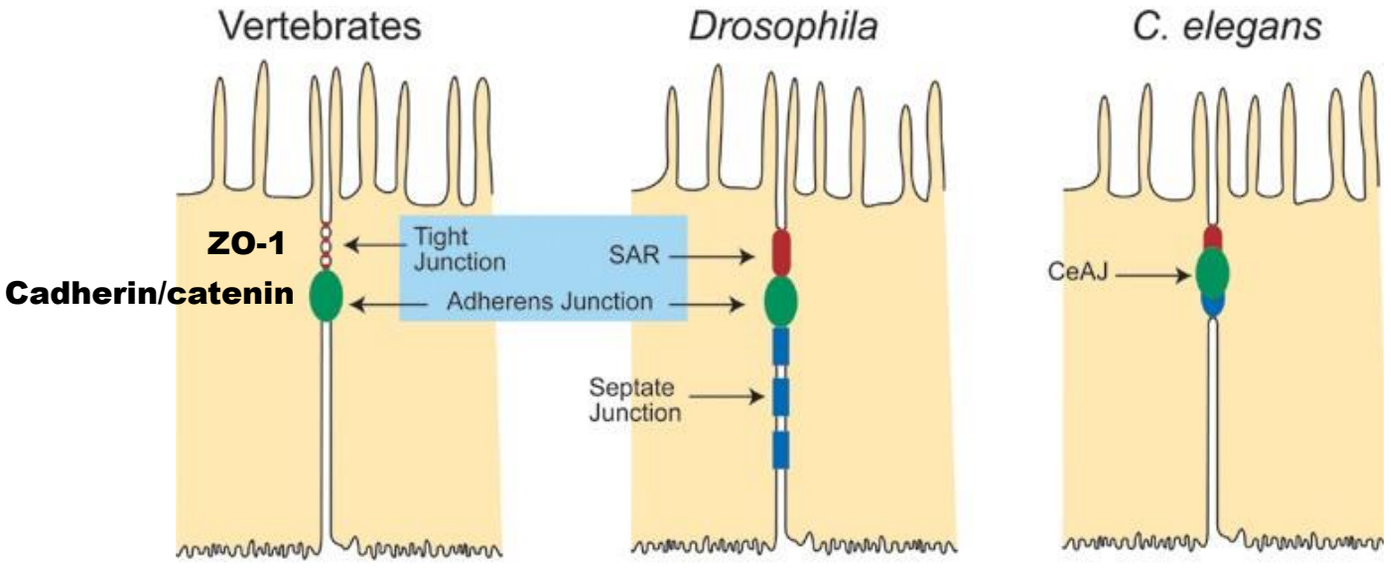




α and β = α/β-catenin

Cronin and DeMali 2021

Cell-Cell junctions differ between cell types



SAR = Sub-apical region junction

Lakkarju 2022

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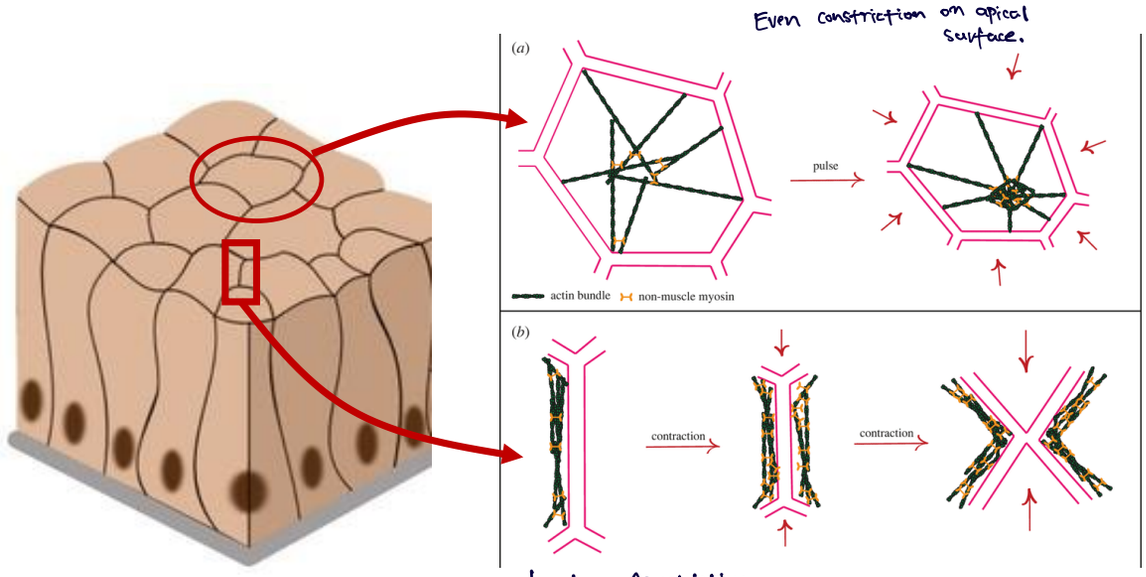
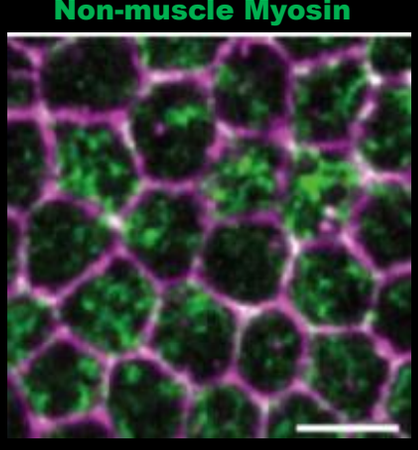
Two common strategies: Medioapical cap (a) versus cell junction (b) activation of actomyosin

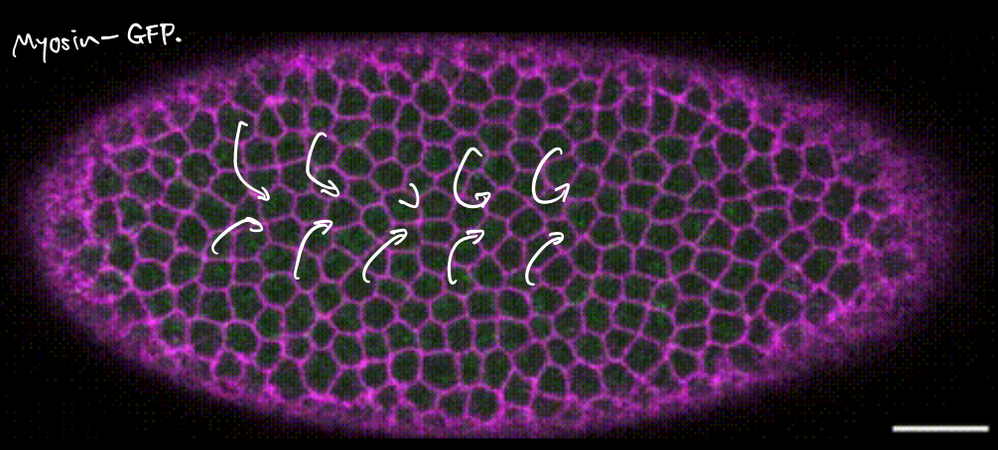


Molnar and Labouesse 2021

Convergence extension

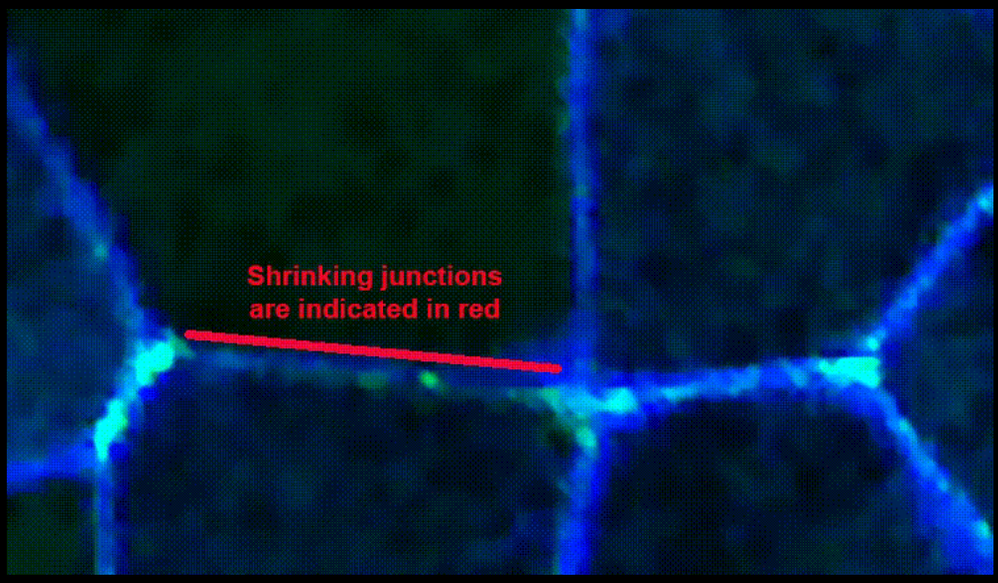
Drosophila ventral furrow cells use medioapical myosin to apically constrict





Mason et al Nat Cell Biol 2013

Xenopus spinal neuroepithelial cells use cortical myosin to constrict their junctions



Peculiarities in different tissues:

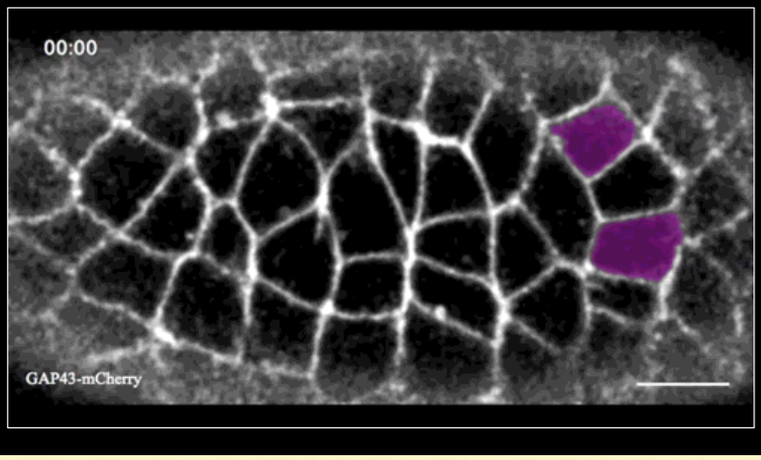
Apical constriction can be directionally-biased By Wnt/Planar Cell Polarity signalling

Rho-ROCK pathway

Pulse to adapt
Stabilise & constrict

Constrictions are pulsatile and ratcheted

Constrictions can be asynchronous or triggered simultaneously



Yan et al 2017

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Apical constriction assessed with static Immunofluorescence dynamic GFP live imaging

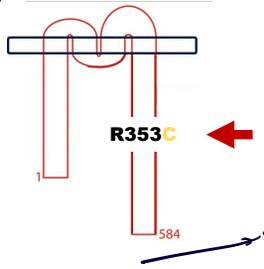
Application example: Identification of a Vangl2 point mutation in a patient who has spina bifida

Spina bifida is caused by failure to closure the embryonic neural tube

Neural tube cells are pseudostratified neuroepithelial cells

CDC

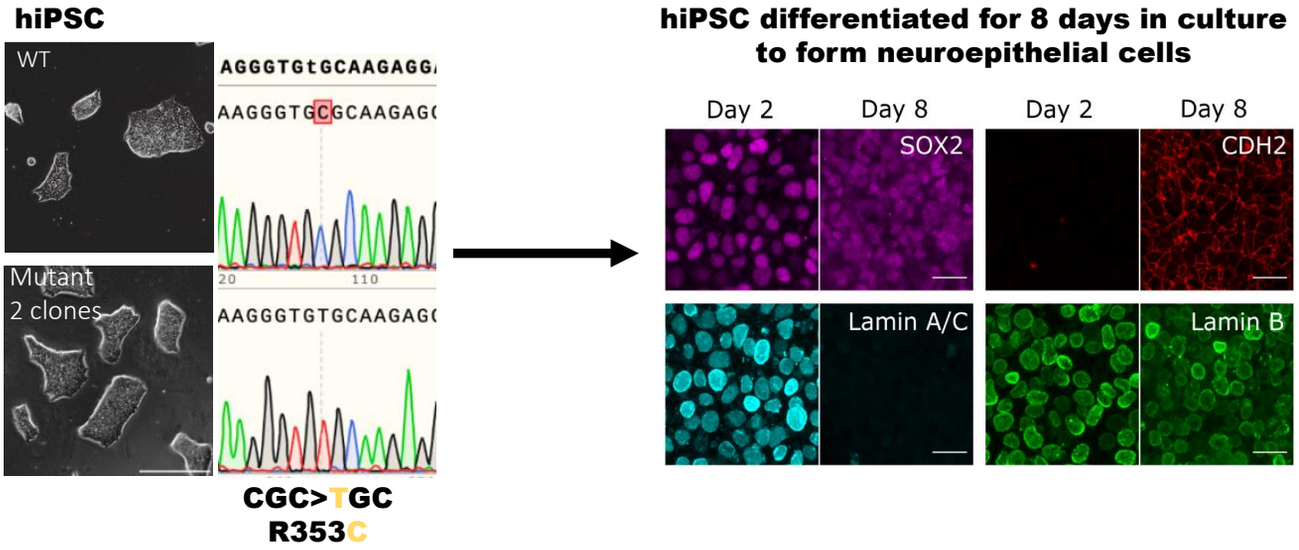
VANGL2 mutation in a patient who has spina bifida:



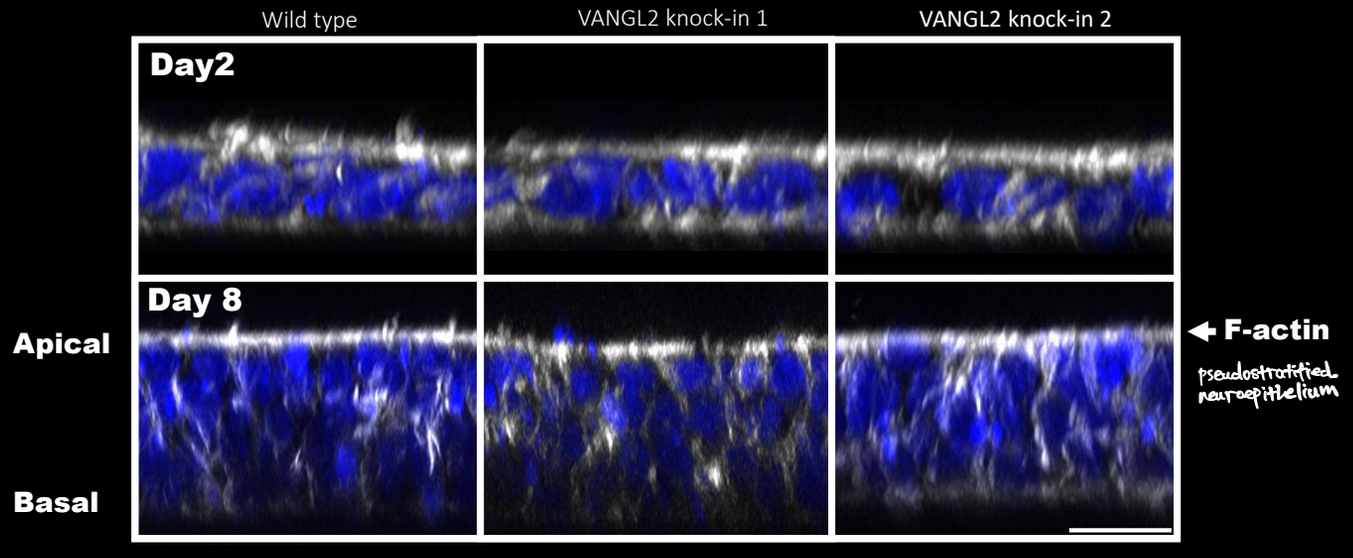
Wnt pathway as well.

VANGL2 is a transmembrane protein up-stream of ROCK

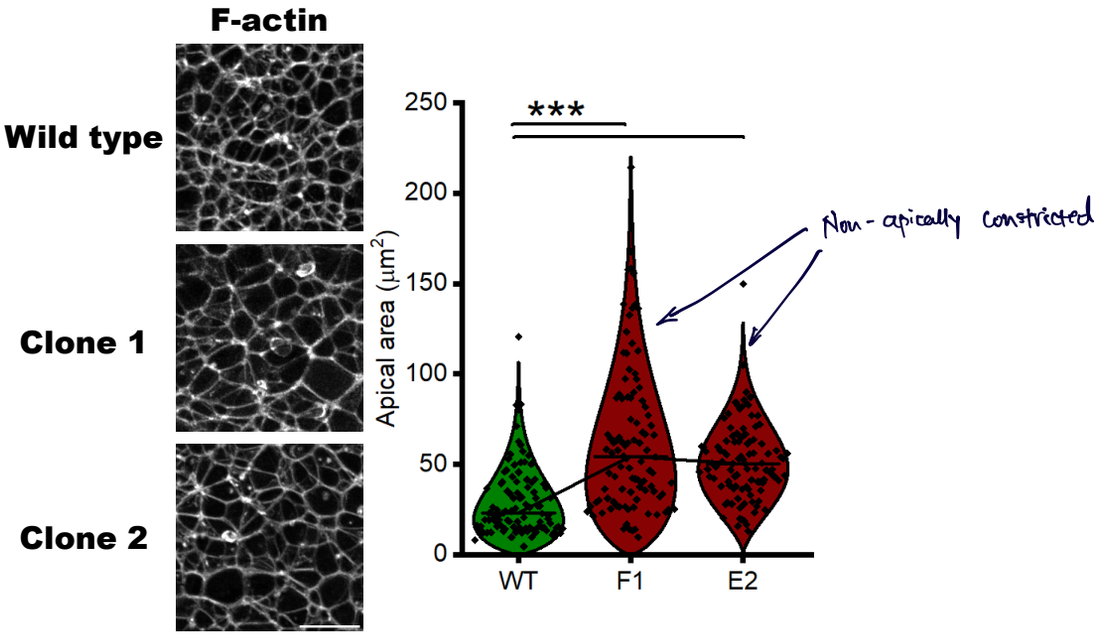
We generated human induced pluripotent stem cell (hiPSC) with the same point mutation using Crispr/Cas9 genome editing



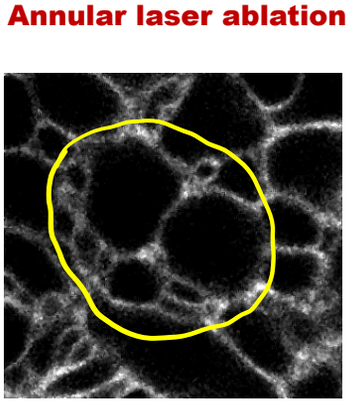
VANGL2 mutation does not change neuroepithelial morphology and apical F-actin



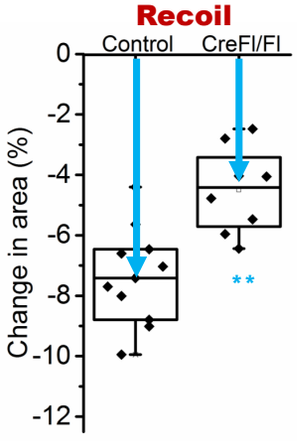
Neuroepithelial cells with the patient point mutation have larger apical areas than controls

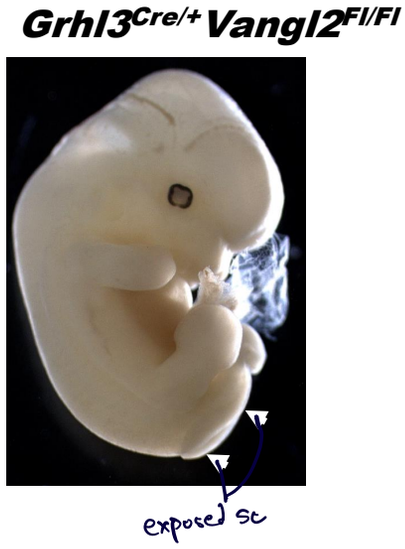


In mice, conditional Vangl2 deletion using Cre/LoxP diminishes neuroepithelial apical constriction and causes spina bifida



Cut around the cells result in constriction indicate tension → Less constriction in mutant.





Vangl2 does not intervene forming, but affect constricting.

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Starting point for further reading:

© 2014. Published by The Company of Biologists Ltd | Development (2014) 141, 1987-1998 doi:10.1242/dev.102228

REVIEW

Apical constriction: themes and variations on a cellular mechanism driving morphogenesis

Adam C. Martin[1,*] and Bob Goldstein[2,*]

Apical Constricti^{o}n

Gabriel Galea

UCL GOS Institute of Child Health

2023

Apical constriction

How epithelial tissue bends. mechanism: Actin-myosin contraction.

Myosin : Myosin II involved in apical constriction. non-muscle type.

Constitute of heavy and light chain. light chain is regulatory.

Activated by Rho-ROCK pathway

$$\text{Rho-GDP} \underset{\text{GAP}}{\overset{\text{GET}}{\rightleftharpoons}} \text{Rho-GTP} \longrightarrow \text{ROCK} \longrightarrow \text{MLC} \rightarrow \text{Actomyosin contract}$$

$$\text{ROCK} \longrightarrow \text{LIMK} \longrightarrow \text{F-actin stabilisation.}$$

Actin : Actin creates structure for myosin pull.

Globular actin (G-actin) : monomers, form triplet & chain in spontaneous manner

Cells have formin, bind to actin dimer, allow preferential polymerisation, form filamentous actin (F-actin)

Arp 2/3 complex bind to actin, allow 70° elongation

Actin in cells

Epithelium: Cortical belt between cell-cell junctions.

Cell-BM junction.

Mesenchyme: Stress fibre between focal adhesions

Lamellipodia & Filopodia.

rays of light speed across wisdom tablecraft
Impressions moving through the Quad
Branches expertly their leaves
White earbuds in coffee.

Apical constriction: Medial apical constriction / Junction

planar-cell polarity pathway affects the direction of contraction.

pulsatile contraction: Contract then adapt to tension.

Apical constriction abnormalities.

Failure to close neural tube → spina bfida

Neural tube made up of pseudostratified epithelium.

Point mutation in Vangl2 → Transmembrane protein upstream of ROCK

↳ Does not change F-actin morphology, affect constriction tension.

Apical constriction:
Involved in formation of the neural tube, changes tissue shape by decreasing the apical surface area

Epithelium: Layer which covers the surface of structures, with cell-cell junctions comparing to cell-ECM junctions in mesenchymal cells

- Components involved in apical constriction:
  - Myosin IIb: non-muscular myosin, contains heavy chain and light regulatory chain
    - Light chain can be phosphorylated to increase activity, allow power stroke of constriction
    - Myosin light chain can be phosphorylated by myosin light chain kinase, dephosphorylated by myosin light chain phosphatase
  - Rho-ROCK signalling: Rho activates ROCK, which activates a series of downstream proteins
    - ROCK can stablise actin polymerisation
    - ROCK phosphorylation of myosin light chain increase actomyosin contractility, promote power stroke.
    - ROCK inhibit microtubule formation
  - F-actin regulators
    - Spontaneous G-actin monomer polymerisation into F-actin is energetically unfavorable so occur at a very slow rate
    - Formin catalyse the reaction, allow linear polymerisation
    - Arp2/3 complex allow polymerisation of actin in a 70° angle to previous polymer.

- Cytockeleton in different cell types:
  - Epithelium: actomyosin bands between AJs, cell-cell contact
  - Mesenchymal: Focal adhesion bind cells with ECM

- Cadherin-catenin complex at the adherence junction
  - E-cadherin bind with E-cadherin from adjacent AJ.
  - Intracellular domain of E-cadherin bind with α/β-catenin, which interact with intracellular actin polymer, allow transmission of force.

<u>Different models of apical constriction</u>
- Formation of actomyosin aggregate in the medial part of the cell, isometeric constriction on all sides
- Lining of actomyosin aggregates along the cell-cell border, allow directional constriction, can be used in convergence & extension (e.g. Xenopus neuroepithelial cells)

Characteristics of apical constriction:
- Asynchronous / signal-induced synchronous constriction
- Contractions are pulsatile, contract followed by stabilisation
- Apical constriction directionality is controlled by Wnt/PCP signalling

- Spinal Bfida: failure of neural tube closure and degeneration of the spinal cord
  - Vangl2 is a co-receptor in the non-canonical Wnt PCP pathway
    - Mutation of Vangl2 R353C single residue mutation lead to severe spina bfida.
    - Vangl2 is upstream of Rho/ROCK signalling in the PCP pathway

- **Experiment:** iPSC constructed neuroepithelium with induced R353C mutation show no morphology changes, but apical constriction ability decreases.
- **Experiment:** Cre/LoxP KO of Vangl2 in mice causes spinal bfida in mice, two-photon laser ablation shows less tension in the apical membrane, less apical constriction.

Apical constriction experiments

Mice iPSC Vangl2 mutation: lack of apical constriction
Mice in vivo Cre-Lox KO + laser ablation of Vangl2: less surface tension due to reduced apical constriction