Cell morphogenesis:

- types of junctions present in different species: mammalians, drosphila C.elegans
  - C elegans contain cadherin-catenin junction.
  - Drosophila contain Adherence junction and septate junction
  - Vertebrates contain Adherence junction, tight junctions, and desmosomes

- ECM: basal membrane + extracellular collagen matrix
  - Basal membrane contains collagen IV and laminin
  - ECM contains collagen I and proteoglycan

Charras & Yap, 2018

AJ junction withstand approx 2~5 pN of tensile force under resting condition, 50~300 pN when pulled

Clarke & Martin, 2021

Actin polymerisation generates 1pN of force to membrane

Myosin contraction creates approx 4pN of tension force, increasing Myo-II contraction or inhibiting arp2/3 polymerisation increase cortical tension.

Apicobasal polarity

Par1, Par2, Par6 used to regulate anterior-posterior axis in C.elegans, apicobasal polarity in vertebrates and drosophila

Role of Cdc42 - Par6 - aPKC axis: regulates AJ position on the apicobasal axis

Experiment

Loss of function mutation in Drosophila of Cdc42 leads to loss of body patterning(denticle lines), delocalisation of apical markers par6, par3 and aPKC, can be rescued with aPKC overexpression - aPKC downstream of Cdc42.

Tepass 2012:

aPKC Par-6 form complex with Par-3 allow polarisation to the apical membrane. Phosphorylation of Par-3/Baz by aPKC weakens the Par-3 - complex interaction, aPKC-Par6 complex move to the apical surface, interact with Crumb, further weakens par3 at AJ, aPKC localise at the marginal zone, AJ in zona adherens.

Par-3 involved in localisation of apical junctions

Experiment

Par3 mutants lead to delocalisation of E-cadherin along apicobasal axis, E-cadherin mutation does not affect par-3 localisation, E-cadherin downstream of Par-3.

Crumb and Scibble antagonistic effects: aPKC phosphorylate Dlg and Lgl, Par-3, limit markers to the basolateral surface

Experiment: KO of Crb and Scrib

Crb KO lead to delocalisation of basal markers, overexpansion of basolateral membrane, KO of scrib lead to delocalisation of apical markers, overexpansion of apical membrane, double KO lead to phenotype similar to scrib mutant, scrib epistatic to Crb.

Planar cell polarity

PCP/non-canonical Wnt signalling: Wnt-Frz-Dsh-Rho/ROCK-MLCK (Myosin contractility) & Rac lamellipodia formation

Experiment:

DN Tcf3 in canonical pathway fail to inhibit PCP pathway, Wnt11 mutant lead to defects in PCP signalling pathway, similar to Rac morpholino oligonucleotide inhibition

Experiment: disruption of the non-canonical Wnt pathway lead to defects in convergent extension in Zebrafish

Dsh zebrafish mutant on the PDZ and DEP domains lead to defects in the PCP pathway.

Wnt11 mutant: silberback

Wnt5 mutant: pipetail

Vangl 2 coreceptor mutant: tribolite

Apical constriction

Gap closure consist of pulsatile apical contraction accompanied by actin stabilisation.

Modes of actomyosin alignments: along the cell-cell contacts (xenopus neuroepithelium), medial actomyosin aggregate isometric contraction of all edges.

Spina Bfida: failure of neural tube closure, degeneration of spinal cord.

Vangl2 R353C single residue mutation lead to spina bfida.

Experiment:

Single point mutation of Vangl2 lead to decreased apical constriction ability in mice iPSC neuroepithelium in vitro.

Using laser ablation to assess surface tension of mice iPSC tissue —> reduced surface tension.

Tissue gap closure mechanism:

In silico simulation shows purse string mechanism and cell movement are both needed during gap closure

Experiment Mole et al., 2019:

GFP tracing + time lapse imaging: allow visualisation of cell-movement and protrusions in the gap.

Cell migration:

Doyle et al., 2021 Particle imaging velocimetry: estimate collagen deformation to reflect stress and strain on ECM, able to visualise 2D and 3D mesenchymal migration dynamics.

O'Neil et al., 2018: Optogenetics: photoactivatable RhoA with subcellular light stimulation induce posterior actomyosin contractions, propelling forward amoeboid migration.

Olguin-Olguin et al., 2021: Photoactivating Rac1 on zebrafish primordial germ cells (PGCs) trigger anterior protrusion formation, while GFP tracing shows retrograde transport of linker protein Esyt2a and Ezrin, the flow can be blocked by dominant negative ROCK.

Olguin-Olguin suggests anterior blebbing inhibit blebbing in other directions, only one protrusion in amoeboid cells. (Self-organising polarisation cascade).

Dona et al., 2013: Life-time reporter by tagging two fluorophores in zebrafish lateral line primordium, shows greater turnover rate of Cxcr4b at the anterior end. Scavenging Cxcr7 receptor generate Cxcl12 a gradient.

Liu et al., 2015: changing culturing condition: different culturing conditions induce different migratory states in tumour cells in vitro. Increased confinement, decrease surface adhesion trigger transition to amoeboid migratory state, while inhibiting RhoA activity lead to mesenchymal transition.

Cell adhesion

EMT prevalence in cancer: single cell EMT rarely found, more often collective cell migration and partial EMT.

Stramer Mayor, 2017: summarises mechanisms CIL, CoA, Chase and run

EMT hypothesised to be related to tissue stiffness:

- Snail transfection into MDMK induce EMT
- Culturing with Sdf-1 initiate migration - not instrinsic.
- Grafting non-migratory NCC to migratory zebrafish - NCC become migratory
- Decreasing stiffness using ablation or inhibition of myosin action: decrease migration
- Increasing stiffness using AFM cantilever or activating myosin light chain increase stiffness and migration (Barriga et al., 2018)
- Fluorescent probe shows increase in N-cadherin, decrease in E-cadherin (EMT) when stiffness is increased.

Hypothesis: increasing in tissue tension is induced by PCP cell movement and gastrulation.

- Barriga et al., 2018: Inhibition of PDZ-DEP domains of Dsh lead to decrease in NCC migration, rescued by increasing cortical tension by pressing cantilever.

Collective cell migration

Scarpa et al., 2015 - CIL: transient formation of N-cadherin cell-cell junction, inhibit Rac-1 and activate RhoA, repolarisation of mesenchymal cells.

Szabo et al., 2016 - In silico simulation shows collective cell migration towards direction is possible when both CIL and CoA effects are present.

Thevenau et al., 2013 - chase and run model: group of placode cell migrate first in zebrafish embryos, NCC population follows. Placode cells release Sdf-1, chemoattractant, NCC then follows the placode population. Blocking of Cxcr4 receptor inhibit chemotaxis of the NCC.

PIV shows changing in traction force orientation of the placode cell moving away from NCC upon contact.

Asymmetric cell division

Sequence of asymmetric cell division: centrosome orientation - cortical flow - asymmetric protein distribution - asymmetric spindle orientation - asymmetric inheritance - differential fate.

C.elegans initial symmetry breaking event: asymmetric distribution of Par6 and Par2.

Lim et al., 2021:

Pole inheriting Par6 become AB cells while pole inheriting Par2 become the P1 cell (Location of sperm entry).

Greater cytoplasmic volume observed in AB cell than P1 cell, P granule aggregate of ~20 proteins observed in the P1 pole.

Hypothesis: centrosome is required for aster microtubule arrangement, lead to asymmetric pulling force and asymmetric inheritance.

Inhibition of CyclinE (centrosome recruitment) result in symmetrical division and synchronous division.

Hypothesis: LIN-5 is required for asymmetric pulling force in C.elegans zygote

Jankele et al., 2015: Optogenetic localisation of LIN5 at both poles lead to symmetrical division. LIN5 and GPR1/2 recruit dynein - retrograde transport of aster microtubule, greater pulling forces.

Extrinsic symmetry breaking mechanism: Wnt signalling, cells asymmetrically divide AWAY from the signal.

Habib et al: Culturing eSC cells in-vitro in contact with Wnt3 containing beads lead to perpendicular division away from the bead, with the cell in contact with the bead expressing pluripotency marker nanog. Cells closer to the Wnt containing beads inherit older centrosomes.

Male drosophila gonad: (Yamashita et al., 2007): Gonadal stem cells closer to the hub (signalling source) tend to inherit older version of centrosome and daughter cells will divide away from the hub. The hub release Dpp and Upd, binds to Tkv and Dome on GSCs.

Older centrosomes have distal appendages - attachment to the plasma membrane

Better at assembling microtubules, more aster spindles, better at assembling cilia.

During drosophila neurogenesis, asymmetric distribution of polarisation markers precedes delamination of the neuroblast. Polarisation of markers occur during metaphase and anaphase, but not during interphase.

Lgl is a basolateral marker, which inhibits aPKC action, inscutable apical marker partners pin, orientates cortical spindles:

Experiment - Lee et al., 2006: Inhibition of Lgl or pin leads to symmetrical division, leading to neuroblast overgrowth.

Determinating neuroblast differentiation: Delta-Notch signalling.

Delta bind to notch of neighbouring cells, stimulate signalling pathway downstream of notch, inhibiting differentiation, inhibit delta expression.

In drosophila, the basal pole inherit basolateral marker Numb, which inhibits Notch expression, and promotes differentiation.

However in vertebrates, GFP tracking shows the cell inheriting the apical domain delaminates and becomes ganglion mother cell, while the cell inheriting basal complex becomes the neuroblast. **(Alexandre et al., 2010).**

Might due to the localisation of Numb at adherence junctions, causes apical pole cell to inhibit notch signalling pathway, induce sister cell to express Notch and retain neuroblast fate.

Epithelial tissue dynamics:

Deletion of intracellular region of the E-cadherin complex lead to defects in gastrulation

Desmosomes: Desmocollin and desmoglein between the cells, intracellular region contains plakophilin and plakogobin, connect to intermediate filaments via adaptor desmoplakin.

Intermediate filaments include keratin, vimentin, nucleofilaments.

Cell-cell interaction studied through foam mechanics, surface tension vs junctional cortical tension Δapex angle

Atomic force microscopy: pressing cantilever to point on cell to assess tension/pressing plane on cell to assess whole cell cortical tension. However this sees the cell as homogenous.

Traction force microscopy: PIV, balanced single cell, opposing if two cells connected, E-cadherin bear stress.
Fluorescence resonance energy transfer: donor + acceptor fluorophore sequences on cells, low FRET in resting junctions, high FRET in desmosomes. High FRET when myosin inhibited, E-cad under constant stress.

Charras lab: assess mechanics of monolayer epithelium: Ca2+ essential for cell-cell connection, EDTA removal lead to fragle tissue. Non-linear stress to strain ratio: larger force first linear, then decrease in stress. Due to keratin straightened and bear tension. DN-keratin desmoplakin mutant lead to fragile monolayer

ECM's role Hypothesis intermediate filaments become stretched and bear tension, act as entropic spring due to the coiled monomer structure of collegen which straighten under stress.
Cells modify the ECM to adapt to stress: increase or decrease collagen production and cross linking
Abnormalities: epidermolysis bullosa / fibrosis and cancer.

Morphogenesis
Local force generation
- Apical constriction + basal expansion
- Cell death and delamination
- AJ reposition

Coordinated force generation
Differential growth
Suzanne lab in France: Folding areas in drosophila embryo have more cell death.
Roeper lab in Cambridge: GFP probing Crb, show radial apical constriction in drosophila salivary gland
Savin et al., 2011: differential growth in length b/e intestine and mesentery, growth of lining tissue in restrictied lumen lead to ridges then villi structures.