KEY POINTS OF DEV3011

SIGNALLING PATHWAYS:

WNT-SIGNALLING PATHWAY

- Inactive (no Wnt signal):
  - Dishevelled is made inactive
  - β-catenin is degraded by a protein complex which consists of CK1 and GSK3 (which are Ser/Thr kinases that phosphorylate β-catenin and degrade it)
    - This protein complex is held together by Axin and APC
  - Wnt target genes therefore remain inactive by the co-repressor Groucho which is bound to LEF1 and TCF
- Active (Wnt signal):
  - Wnt binds to Frizzled and LRP
  - Dishevelled is made active
  - CK1 and GSK3 (from the protein complex) phosphorylate LRP activating it
  - Axin is therefore recruited to the activated LRP, disrupting the degradation complex
  - β-catenin therefore doesn't get phosphorylate/degraded d and accumulates in the cytoplasm
  - β-catenin enters the nucleus and binds to LEF1/TCF, which removes Groucho
  - This allows transcription of Wnt target genes
- Regulation of Wnt-signalling:
  - Extracellular regulators:
    - Wnt Inhibitory factors such as secreted frizzled related protein (sFRP)
    - Dickkopf and Wise which down-regulate LRP
  - Intracellular regulators:
    - LiCl which inhibits GSK3 - therefore β-catenin avoids degradation
- Examples of Wnt signalling in development:
  - Wnt9b: is required for normal kidney and reproductive tract development

SONIC-HEDGEHOG (Shh) SIGNALLING PATHWAY:

- Inactive:
  - With no Shh signal, the Patched receptor inhibits Smoothened
  - This allows the inhibitor SuFu to hold the Gli transcription factors close to the microtubules allowing PKA and Slimb to act on the GLIs (Ci) causing them to take a form that represses Shh transcription
  - The GLIs are also phosphorylated/degraded
  - Shh target genes are therefore repressed
- Active:
  - Shh binds to the Patched receptor which stops the inhibition of Smoothened
  - Smoothened inhibits PKA and Slimb allowing the GLI transcription factors to be released from the microtubule/SuFu (and not be phosphorylated)
  - The GLI transcription factors can then enter the nucleus and activate Shh target genes and ultimately remove the repression of Gli3
- Examples of Shh signalling in development:
  - Shh is required for digit formation in limb development (anterior-posterior)

RTK (RECEPTOR TYROSINE KINASE) SIGNALLING PATHWAY:

- How it works:
  - Ligand comes along, bringing two of the receptors into close proximity
  - The receptors DIMERISE and the activated tyrosine kinase domains will CROSS-PHOSPHORYLATE each other leading to activation and further intracellular signalling
  - Proteins with SH2 domains bind to the phosphorylated tyrosines
  - RTK phosphorylates GNRP (guanine nucleotide releasing factor) which phosphorylates RAS which phosphorylates RAF which phosphorylates MEK
  - MEK then enters the nucleus where ERK phosphorylates the inactive transcription factor (ITF) to make it active so it can modulate transcription
  - (RTK >> GNRP >> RAS >> RAF >> MEK >>(nucleus)>> ERK>> ITF made active)

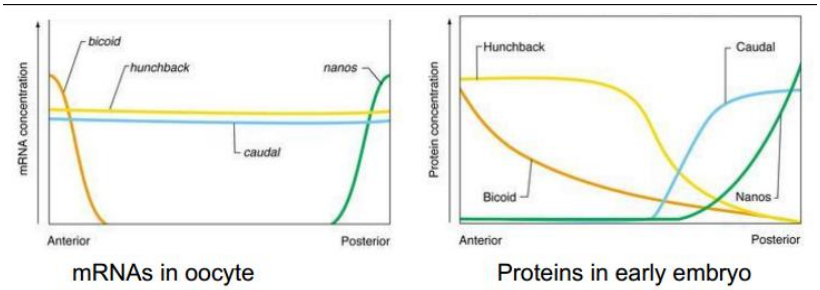
TGF-β SIGNALLING PATHWAY (TGF-β/Activin/BMP):

- How it works:
- The Activin (or TGF-β/BMP) binds to the receptor
- These receptors dimerise and crossphosphorylation occurs between the Ser/Thr kinase domains
- When the Activin/TGF-β has bound, the phosphorylation signal directs down into Smad 2,3 which are transcription factors
  - For BMP, the signal directs down to Smad 1,5,8 instead.
- The Smad's become phosphorylated and bind to Smad 4
- Smad 4 enters the nucleus and binds to DNA, altering gene expression via transcription of repression
- The ligands of the TGF-β pathway act as morphogens
- TGF-β signalling is critical for germline specification (BMP4 KO exhibits PGC loss)

ANTERIOR-POSTERIOR/ LEFT-RIGHT/ DORSAL-VENTRAL PATTERNING:

DROSOPHILA:

- Anterior-Posterior (Head to Tail):
- The maternal mRNAs establish the A-P axis
- Bicoid and Hunchback are critical for anterior formation (before fertilisation)
- Nanos and Caudal are critical for posterior formation (before fertilisation)



mRNAs in oocyte Proteins in early embryo

- Upon fertilisation, these mRNAs are translated (Protein products can diffuse from site of production because the zygote is a syncytium)
- Upon fertilisation, protein concentration gradients are established (which are morphogens)
- Bicoid concentration remains high at anterior end
  - Nanos concentration remains high at posterior end
- Bicoid inhibits translation of caudal at the anterior end (and activates Hunchback)
  - Nanos inhibits translation of hunchback at posterior end (and activates Caudal)
- All these proteins are transcription factors which control zygotic gene expression and ultimately regulate the formation of anterior/posterior pattern formation
- As well as maternal genes, there are then ZYGOTIC GENES (A-P segmentation):
  - Gap genes - divide embryos into broad A/P domains
  - Pair-rule genes - define 14 parasegments
  - Segment polarity genes - pattern the parasegments and divide them into compartments
  - Selector (homeotic) genes - specify segment identity(Hox genes)

XENOPUS:

- Dorsal-Ventral:
- The polarity of the egg is determined before it is fertilised with an animal and vegetal pole being present (many maternal factors in the vegetal half)
- The DORSAL side of the Xenopus embryo develops OPPOSITE THE SITE OF SPERM ENTRY
- Sperm enters the animal hemisphere >> causes the outer cortex to loosen
- CORTICAL ROTATION then occurs - this involves microtubules becoming oriented away from the site of sperm entry. It results in the movement of DORSALISING FACTORS to the opposite side of sperm entry (via microtubules)
- Dorsalising factors include:
  - Dishevelled - acts by binding to GBP and Kinesin. This causes the inhibition of GSK3 resulting in stabilisation of β-catenin and activation of Wnt signalling