BMP Signalling Pathway

- Bone morphogenetic proteins (BMPs) are a group of secreted signalling molecules that belong to the Transforming Growth Factor β (TGF-β) family of proteins
- BMPs induce bone formation and regulate embryonic development & adult tissue homeostasis
- BMPs are involved in cell proliferation, differentiation and apoptosis
- BMP signalling is regulated extracellularly and intracellularly by a complex network of ligands, receptors, agonists and antagonists

Signal Transduction

- BMPs initiate a signal transduction cascade by binding to enzyme-linked cell surface receptors
- There are 2 classes of BMP receptor proteins (each have a serine-threonine kinase domain); **type I** and **type II**
- BMP binding promotes formation of heterotetrametric receptor complex comprised of 2 copies of type I receptor and 2 copies of type II receptor
- Type II receptor phosphorylates the type I receptor
- The activated type I receptor directly binds and phosphorylates an intracellular Smad protein
- The phosphorylated Smad protein binds to the co-mediator Smad4 to form a transcriptional regulatory complex
- This regulatory complex enters the nucleus where it functions as a transcriptional factor to either activate or repress target genes (other cofactors can be involved)
- R-Smads involved in BMP signal transduction include Smad1, Smad5 and Smad 8

Nucleocytoplasmic Shuttling

- Smad proteins are shuttled between the nucleus and cytoplasm
- In the nucleus, R-Smad is dephosphorylated and disassociates from the Smad4 complex
- R-Smad and Smad 4 monomers are exported back to the cytoplasm
- If BMP receptor is still active, R-Smad will be rapidly phosphorylated and reassociate with Smad4
- The active Smad complex will relocalise to the nucleus and the cycle repeats
- Nuclear translocation of Smad proteins is dependent on continuous activation of the BMP receptor complex
- If receptor is no longer active, then Smad4 will accumulate in the cytoplasm
- This allows the cell to rapidly respond to changes in BMP receptor activation and ensures that the R-Smad and Smad4 complexes remain in the nucleus for the same period of time the receptors are activated

Limb Formation in Vertebrates

- Involves morphogenetic events including changes in cell shape, increase in rate of cell proliferation and occurrence of discrete and localised cell death

Interdigital Cell Death

- Programmed cell death (apoptosis) is important for limb development and helps establish the shape and morphology of digits and joints
- Pattern of cell death is precise, genetically programmed and strictly regulated at a molecular level
- Variation in temporal and spatial pattern of cell death during embryogenesis can result in major differences in limb morphology
- Interdigital cell death results in digit separation

eg. in species with free digits such as chickens and humans, interdigital cell death eliminates the tissue between the developing digits, allowing them to move freely and independently of each other

- In species with webbed digits, such as ducks, minimal cell death occurs in the interdigital region so the tissues between the digits remains
- Activation of BMP signalling pathway in interdigital cells triggers programmed cell death

Limb Development

- In early embryogenesis, vertebrates develop primitive paddle shaped limbs
- Functional limb structures are produced through proliferation, changes in cell shape and programmed cell death

Syndactyly

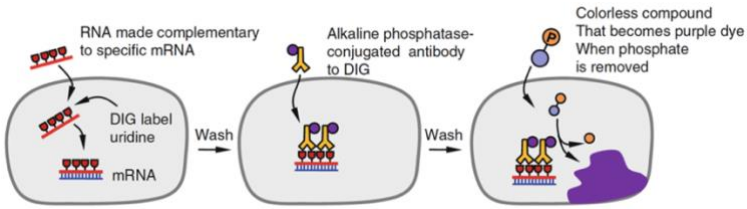
- **Syndactyly** refers to digits that are interconnected by webbing
- Occurs naturally in species such as ducks and otters
- Common malformation in humans (1 in 2000)
- Molecular mechanisms that control interdigital cell death and syndactyly are highly conserved in vertebrates

Neutral Red Staining

- **Neutral red** is a vital dye (can be applied to live cells without killing them) that is readily absorbed and endocytosed by cells and internalised within the lysosome
- Healthy cells stain a light pink colour, apoptotic cells stain a darker red

In Situ Hybridisation

- ***In situ* hybridisation** detects nucleic acids within specimens
- Provides information about the temporospatial pattern of gene expression
- mRNA transcripts are detected using *in situ* hybridisation probes that bind to target mRNA via complementary base pairing
- the bound probe is then detected using an antibody conjugated to the enzyme alkaline phosphatase
- alkaline phosphatase converts a colourless compound to a purple compound that can be easily visualised

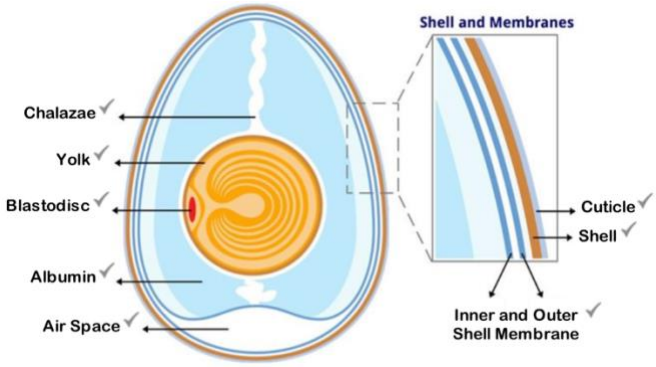


Vertebrate Development

- **Phylotypic stage** is the early stage in development where all embryos have a similar structure and appearance
- Early events in embryo development are highly conserved (remain unchanged)
- As the vertebrate develops, the embryo differentiates and becomes species-specific

Chick Egg Anatomy

- **Chalazae** holds the yolk in place by attaching to the shell membrane
- **Yolk** is bound by a thin membrane and contains nutrients for the embryo
- **Blastodisc** is the site of fertilisation and embryo development
- **Albumin** protects the yolk and is a source of nutrients
- **Air space** is the air-filled pocket between the inner and outer shell membrane
- **Cuticle** is the outermost coating of the egg, protects from bacteria and dust
- **Egg shell** is the semi-permeable barrier



- The brain and nervous system is early chick embryos is primitive and non-functional
- Brain develops 3 subdivisions (forebrain, midbrain and hindbrain) that look like bulges
- Brian becomes functional during mid-gestation
- **Somites** are paired blocks of cells along the length of the embryo that give rise to the vertebral column, ribs, skeletal muscles and connective tissue
- The **heart** and circulatory system develop rapidly in chick embryos and are the first organs to become functional (heart starts beating & pumping blood at E2.5)
- The **amnion** is a membrane that encloses and protects the embryo and the amniotic fluid that surrounds it
- Developing eye is a darkly pigmented outgrowth from the forebrain
- The **tail bud** is responsible for elongation of the embryo and gives rise to the vertebral tail (all species have a tail bud)
- The **allantois** is an extraembryonic membrane that stores waste and helps with gas exchange (increases in size throughout gestation)
- **Leg buds** are a pair of bulges in the caudal region of the embryo that give rise to the hindlimbs
- **Wing buds** are a pair of bulges located hallway down the embryo and give rise to the chick wings