Discuss the evolution of the tetrapod limb from a fin to the wide variety of limb forms seen today. Include a discussion on the morphological and molecular changes that have had to evolved.

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Tetrapod translated from Greek means four feet and includes a wide variety of living and extinct animals including amphibians, reptiles, dinosaurs, birds and mammals. According to the fossil record tetrapod limbs evolved from sarcopterygian fish's lobe-fins, in the Devonian period approximately 370 million years ago, this fin-to-limb transition is a key evolutionary event that allowed life on land. Further evolution allowed for the wide variety of limb forms (or absences) seen today on swimming whales, walking humans, flying bats and slithering snakes (one of the exceptions to the four-footed tetrapod category). Those that kept their limbs generally maintain the homologous bone layout of one long bond (humerus) attached to two long bones (radius and ulner) attached to multiple (usually shorter) bones (carpals, metacarpals and phalanges). This essay will give an overview limb development before discussing this fin-to-limb transition and further specific evolution with the morphological and molecular changes that have had to evolve to result in these changes.

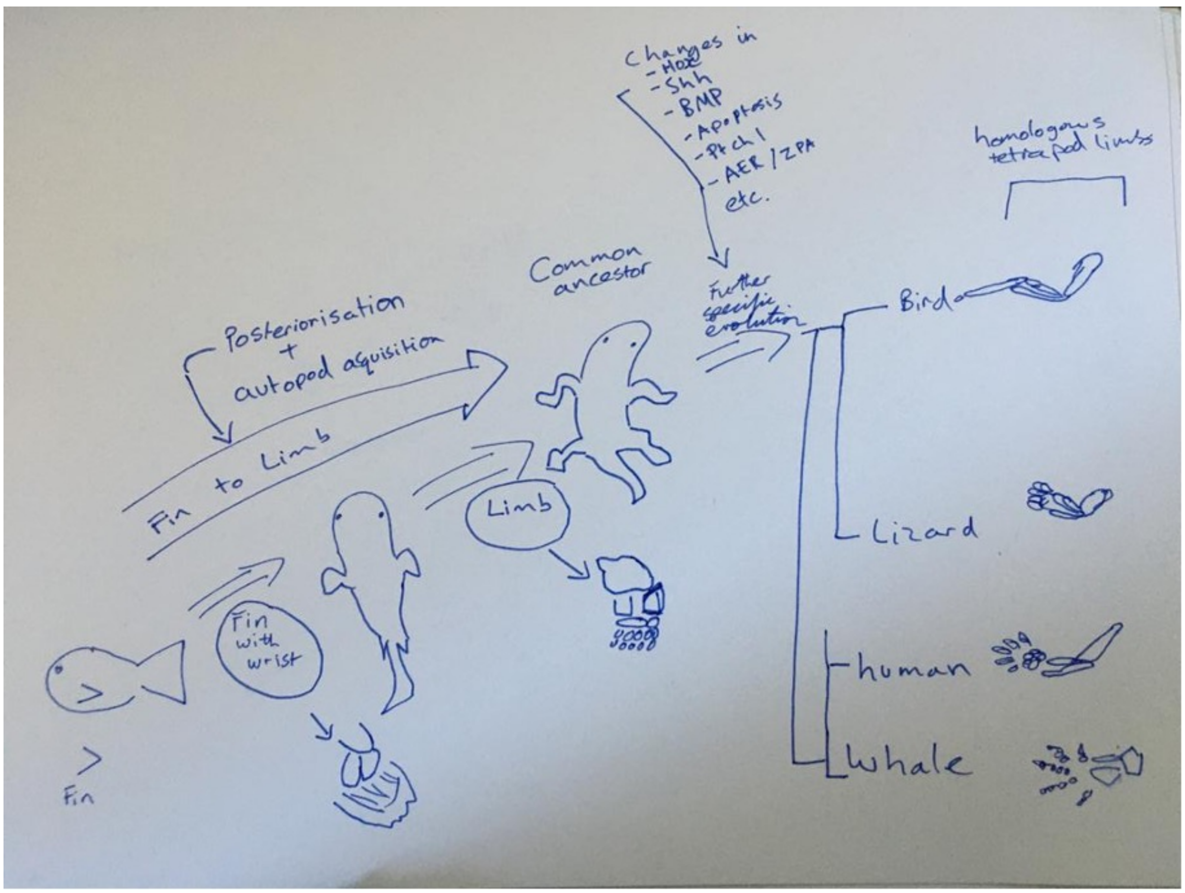

Figure 1: Fin to Limb Overview.

Limbs form in a standard way for tetrapods with the Zone of Polarising Activity (ZPA) determining the anterior-postiror axis and Apical Ectodermal Ridge (AER) determining the proximo-distal axis, these (ZPA and AER) signal to each other throughout limb development to form a fully functioning limb. The ZPA when cut out and grafted into the opposite side forms double posterior duplication showing that the ZPA is involved in digit formation. Sonic Hedgehog (shh) is the polarising factor of the ZPA and if you put a protein implant in the same location as the grafted ZPA it shows the same mirror image duplication proving that it is the molecule from the ZPA that has been show to work in a gradient with the high concentration of shh giving rise to the little finger and low giving you your thumb. The AER is involved in proximal to distal outgrowth of the limb and this has been shown by taking the AER off during chick development. The earlier it is taken off the less distal structures are formed and the later you take it off the more distal structures are formed, the humerus is formed before the radius and ulna which are formed before the digits. We also know that fibroblast growth factors are the signalling proteins for this as beads soaked in FGF8 can replace the AER when removed to allow for normal proximal to distal outgrowth. In situ hybridisation looking at mRNA has shown shh and FGF8 locations (see sketch below) and where they overlap FGF induces shh and shh maintains FGF creating the feedback loop required for normal development. Limb positioning is determined by Hox gene expression, specifically where Hoxb-8 is strongest is where the ZPA is formed, this was shown through retinoid receptor antagonist being applied to presumptive region downregulating Hoxb-8 expression. Tbx genes are also involved in fore and hindlimb patterning with Tbx5 and Tbx4 triggering limb initiation for fore and hindlimbs respectively, by activating the Wnt/FGF signalling cascade, We do not fully understand limb development and multiple models for limb patterning have been proposed these include the; Progress Zone model, differentiation Wave-Front Model, and Turing model, but it is important to note that this molecular aspect are conserved across tertrapods.

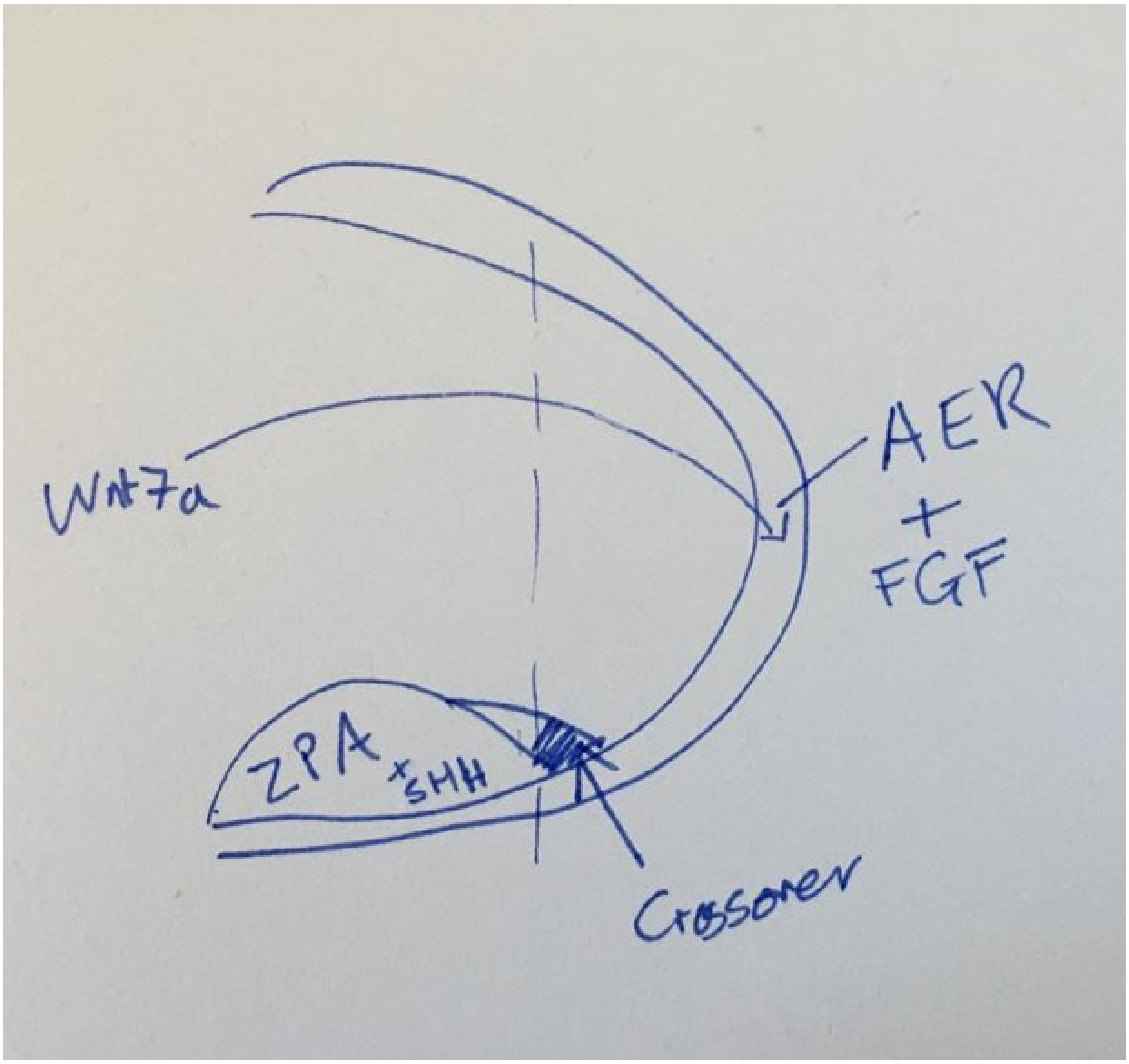

Figure 2: A figure to show the standard formation of limb development for tetrapods with the Zone of Polarising Activity (ZPA) determining the anterior-postiror axis and Apical Ectodermal Ridge (AER) determining the proximo-distal axis, these (ZPA and AER) signal to each other throughout limb development to form a fully functioning limb.

The key to fin-to-limb transition is the acquisition of the autopod, which is the most distal part of the tetrapod limb, and posterioirsation, which is the reduction of anterior structures. Fish have an AER but it folds over on itself and proliferates making the fin rays, but fish only have early phase hox gene expression while tetrapods have an early and late phase hox gene expression which allows for longer AER functioning and therefore acquisition of a hand plate. Knocking out hox genes linked to this later phase shows that it does not form these distal sections as seen in Hoxa13 and Hoxd13 knock outs where this autopod does not form. And interestingly if these are not knocked out but Hoxa11 and Hoxd11 are knocked out the hand plate has been shown to form normally but the radius and ulnar do not form correctly. Subdivision of Hoxa11 and a13 domains with the increase of shh have been shown to be linked to this acquisition of the autopod. Postioroisation has been linked to changes in Gli3 expression, which appears to be connected to elongating the digital skeleton and defining the autopodal area as well as also being linked to Hand2 a key gene in limb bud. 5'Hoxd expression increases shh transcription therefore increasing its signalling which should also contribute to posteriorisation. And the loss of this AER folding over on itself most likely also contributes to posteriorisation as this folding has been seen in the fossil record and experiments in shark fins.

Further specific evolution come from differential growth, changes in chrondrogenesis, changes in cell death, and changes in limb positioning. Varying expression of hox genes, shh, FGF etc as well as timings of these depending on the species leads to differentiation in limbs. Horses have one elongated and fused digit, and bats have elongated digits with skin flaps to allow for flight. There are different methods of reducing digits in tetrapods; these are (1) shh expression, which has been observed in skinks and the difference between 5 toed and 2 toed skinks is premature termination of shh. (2) interdigital apoptosis, which is the horse example where BMP-induced apoptosis is greatly increased in the interdigit mesenchyme when comparing horses to mice limb bud development. (3) Ptch1 expression, which when reduced due to a degenerated limb bud-specific cis-regulatory module (LRM) results in the reduction of Ptch1 expression in the mesenchyme causing a loss of asymmetry causing even toes that are artiodactyl we see in bovine and pig limbs. BMP2 expression and bmp signalling are increased in bat forelimbs in comparison to their hindlimbs and has been seen to increase cartilage proliferation. And altering BMP expression can cause or remove webbing in duck and chicken feet. Limbs can be completely removed, as in snakes (pythons) where overexpression of flank genes Hoxc-6 and Hoxc-8 can prevent limb bud initiation and when snakes are manipulated they can grow limbs, meaning they lost their ability to make limbs. Conversely you can prevent chicken limb bud induction through overexpression of these flank hox genes (hoxc-6 and hoxc-8). This idea of inducing limbs can be applied to making more digits like in polydactyl in which overexpression of SHH has been connected.

In conclusion fin-to-limb was the first evolutionary step that allowed animals to live on land as well as the water, but further evolutionary changes in the ZPA and AER as well as hox gene expression along with Ptch and bmps have allowed for the great variety of tetrapod limbs we see today.