Regulation of Chondrogenesis and Osteogenesis

- Sox9 → PTHrP
- PTHrP gene → PTHrP
- Sox9
- Ihh
- Runx2 (Cbfa)
- OSX
- Bone

Mesenchymal stem cells → chondroprogenitors → chondroblasts → chondrocytes

Proliferating chondrocytes → hypertrophic chondrocytes → osteoblasts → osteocytes
Supplementary figure 4  Summary of the expression pattern similarities and differences between fishes and tetrapods.

Zebrafish sox9a acts upstream of pthlha in many cartilages of the craniofacial region and in the pectoral fin. This is consistent with the known role of sox9a in development of osteo-chondroprogenitor cells, including the cartilaginous pharyngeal skeleton. In pthlha knockdown embryos, runx2b transcript is up-regulated, which is consistent with the hypothesis that pthlha knockdown leads to premature alizarin staining in the ceratohyal bone collar, suggesting that Pthlha in zebrafish is necessary to slow chondrocyte maturation. Thus, our results suggest that the role of pthlha in zebrafish chondrogenic and osteogenic pathways conserves the role of Pthlh in mammals.

The regulation of sox9 by Pthlh showed considerable conservation between zebrafish and mammals. The increased expression of runx2b in knockdown Pthlha animals suggests that Pthlh may have a direct role in down-regulating runx2b expression. Our data are consistent with the hypothesis that Pthlh decreases the expression of runx2, which then retards bone mineralization. In the pthlha knockdown animals, runx2a is upregulated, which leads to premature bone mineralization.

In mammals, Runx2 controls osteoblast differentiation by binding the promoter of Osx (osterix), which then regulates the production of bone matrix proteins including Col1a2 and Sparc (alias osteonectin) (Rotllant et al. 2008) and this regulatory cascade appears to be conserved in zebrafish. Zebrafish Pthlh knockdown animals showed a reduction in osx expression in the neurocranium, pharyngeal arches, and pectoral girdle similar to that in humans with campomelic dysplasia (Yan et al. 2002). Thus the data indicate that zebrafish and mammals show conservation during bone development as well as a conserved mechanism for bone diseases.

With the observation that fishes express duplicated gene pathways that often regulate orthologous structures and systems in mammals, additional studies are necessary to understand how the gene pathways evolved. For example, do the co-orthologs use different combinations such that there are parallel sets of cells using sox9a, runx2a and pthlha while other use sox9b, runx2b and pthlhb (or a some other specific combination), or are there more intricate interactions during patterning.