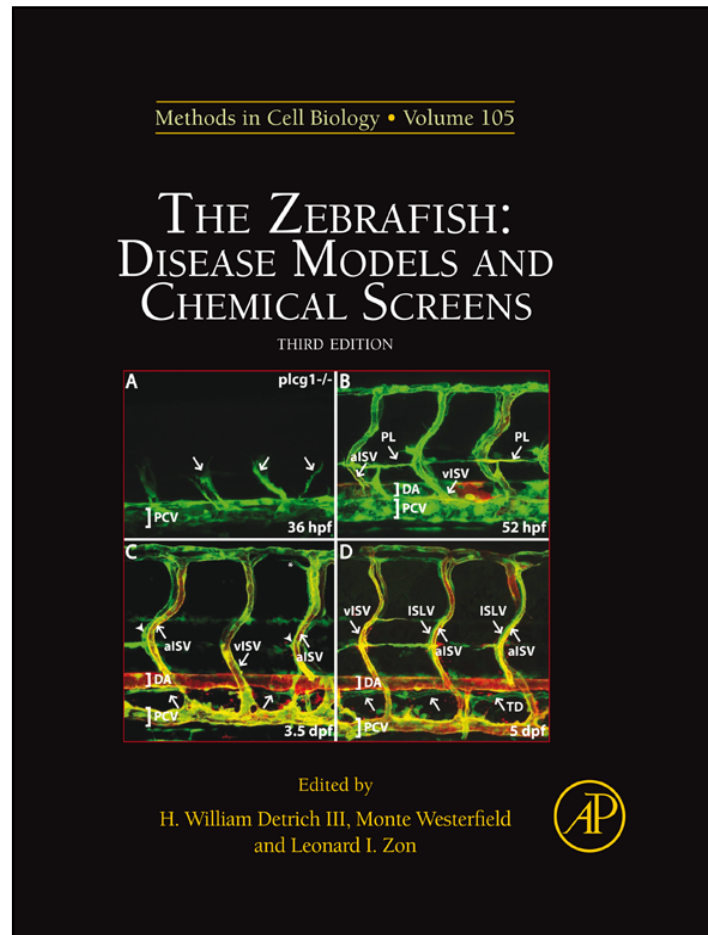


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**CHAPTER 10**

# Not All Bones are Created Equal – Using Zebrafish and Other Teleost Species in Osteogenesis Research

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## Abstract

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  - II. The Evolution of Skeletal Tissues
  - III. Cartilage and Bone in Teleost Fish
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## Abstract

Developmental osteogenesis and pathologies of mineralized tissues are areas of intense investigations in the mammalian field, but different from other areas of organ formation and developmental biology, zebrafish have been somewhat slow in joining the area of bone research. In recent years, however, genetic screens have provided a number of exciting mutants, and transgenic lines have been developed that permit visualization of osteoblasts and osteoclasts *in vivo*. We here review some of the

recent literature and provide examples where insights from studies in zebrafish have complemented the information available from mammalian models or clinical studies. Furthermore, we provide a comparative overview about different forms of bone within the teleost lineage, and between teleosts and mammals.

The vertebrate skeleton serves numerous functions, most notably by providing a stable, but mobile framework against which muscles act. The endoskeleton also has protective functions for the brain and many internal organs, and serves as a store for minerals. The postcranial endoskeleton consists of the axial skeleton, supporting the main body axis, and the appendicular skeleton, supporting the extremities of the body. Bone and cartilage are the main components of the endoskeleton, being produced by osteoblasts and chondrocytes, respectively.

Given the vital roles of the skeleton, it is not surprising that in the human clinic a number of diseases and pathologies affect the skeletal system. They include metabolic bone diseases such as osteoporosis and osteoarthritis as well as birth defects such as cleft palate, congenital vertebral malformations, or skeletal dysplasia (for reviews, see McInnes and O'Dell, 2010; Ralston and Uitterlinden, 2010; Zelzer and Olsen, 2003). Particularly osteoporosis (an increased risk of bone fracture due to a decrease in bone density) and osteoarthritis (a degenerative disease affecting joints) are extremely common, and have an enormous bearing on health care costs in an ever-aging society.

In many areas of cell biology and organogenesis, zebrafish (*Danio rerio*) have provided a plethora of useful models that can be employed to study processes with relevance to human disease. In contrast to mouse and chicken, however, zebrafish has a short history as model for bone disease research, or, for that matter, for studying bone formation from a developmental or cellular point of view. Part of this is certainly due to historic reasons. Initially zebrafish were mainly used to understand early developmental processes, and only gradually the use of the system has been expanded to areas of organogenesis and larval development. Another reason is that there is a diffuse notion about teleost bone being “weird” and different from “normal” (i.e., mammalian) bone. Within the vertebrate phylum, teleost species are by far the most successful (at least in terms of species number), so one could actually argue what “normal” is. However, these discussions are, to a degree, doomed to be pointless in an anthropocentric funding environment.

Despite a slow start, in recent years a steadily raising number of publications prove the zebrafish as a valuable complementation to the traditional model organisms. As so often before, the availability of mutants has been a driving force in the utilization of zebrafish, and forward genetic screens have already yielded hundreds of mutants (reviewed by Spoorendonk *et al.*, 2010). Fortuitously, some zebrafish mutants survive far longer than knockouts in their murine orthologues (see below), allowing to obtain additional information even in cases where mouse mutants had been available for a number of years. An additional and exciting opportunity for studying skeletal formation and osteogenesis in fish is the possibility to observe bone-forming cells (osteoblasts) *in vivo*. Transgenic lines for a number of informative markers have been generated by now in zebrafish and medaka (*Oryzias latipes*) (Renn and

Winkler, 2009; Spoorendonk *et al.*, 2008). This is a key advantage of using zebrafish and medaka as model organisms.

Among the bone and osteogenesis mutants that have been studied in zebrafish are some instructive examples, two of which are discussed in more detail below. Furthermore, with this chapter, we provide an overview about the different types of skeletal tissues that are present in zebrafish, medaka, and other teleost species.

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## I. Case Studies – Using Zebrafish for Addressing Biomedical Questions

An attractive case for the zebrafish as a bone disease model was recently demonstrated by Clement *et al.* (2008). The authors presented *in vivo* evidence in support of a loss of heterozygosity (LOH) model in osteochondroma formation, which is associated with hereditary, multiple osteochondromas syndrome (MO, also known as hereditary multiple exostoses). Until recently it has been discussed in the field whether LOH or a gene dosage effect due to haploinsufficiency is responsible for osteochondroma formation as LOH could not be detected in all osteochondromas (Bovee *et al.*, 2010).

Particularly, the majority of the patients suffering from MO carry mutations in either of the exostosin genes EXT1 or EXT2 (Jennes *et al.*, 2009), two genes involved in heparan sulfate (HS) biosynthesis. Clement *et al.* (2008) introduced the zebrafish mutant *dackel* (*dak*), harboring a mutation in *Exostin2* as a model with an osteochondroma-like cartilage phenotype: in these mutants, chondrocytes have a round shape and form clusters of cells instead of being flattened and aligned in columns.

Use of the mouse as a model for this disease has turned out to be nontrivial since both *Ext1*<sup>-/-</sup> and *Ext2*<sup>-/-</sup> mice die during early development (Lin *et al.*, 2000; Stickens *et al.*, 2005), and mice heterozygous for *Ext1* (Hilton *et al.*, 2005) or for *Ext2* (Stickens *et al.*, 2005) do not form osteochondroma-like structures or, respectively, do not form those in long bones as in human patients.

Carrying out transplantation experiments of *dak*<sup>-/-</sup> cells into wild-type embryos, Clement *et al.* (2008) were the first to show that in some cases *dak*<sup>-/-</sup> cells are not rescued by HS secreted from the surrounding cells and can initiate outgrowths similar to human exostoses. This finding is in support of an LOH model and has recently been independently confirmed by two new mouse models for osteochondromagenesis (Jones *et al.*, 2010; Matsumoto *et al.*, 2010) in which the authors by different strategies generated chondrocytes with a chimeric LOH genotype for *Ext1*.

Apparent advantages in this study were: (1) that zebrafish mutants often survive to stages that allow investigation of bone phenotypes, whereas corresponding murine models die during earlier stages of development, and (2) that transplantation experiments to create mosaic animals can be carried out with relative ease. In addition, by phenotypic comparison of *dak* mutants with mutants isolated from a forward genetic

screen, the authors could introduce 3'-phosphoadenosine 5'-phosphosulfate transporter (*papst1*) as a gene involved in HS synthesis. Thus, *papst1* represents a potential candidate in cases of patients where no mutations in the exostosin genes can be detected. One mechanism by which HSs are thought to act on chondrocytes is by restricting the diffusion of factors such as Hedgehog (Hh) ligands in the growth plate (Koziel *et al.*, 2004). The importance of the Hh pathway is well established for skeletal development and Indian hedgehog (*Ihh*), a member of the Hh family of secreted ligands, is expressed in chondrocytes. There is evidence that *Ihh* controls chondrocyte proliferation and osteoblast differentiation (Lai and Mitchell, 2005; St-Jacques *et al.*, 1999).

However, some details of the Hh pathway and particularly its effect on bone mass after initial stages of development are still not well understood. Recently two studies analyzing bone homeostasis in mice with reduced Hh signaling resulted in contradicting findings. One group (Mak *et al.*, 2008) found that conditional deletion of the Hh receptor Patched1 (*Ptch1*) (deletion of which leads to increased Hh signaling) in mature osteoblasts, using an *Osteocalcin*-Cre line, leads to an overall reduction in bone mass. The authors could relate this to increased osteoclastogenesis, induced via higher *PTHrP* and *RANKL* expression in osteoblasts. Another group (Ohba *et al.*, 2008) investigated a mouse model haploinsufficient for *Ptch1*, which exhibited a high bone mass phenotype. The authors could also measure increased bone mass in patients with nevoid basal cell carcinoma (NBCCS or Gorlin syndrome), a consequence of *Ptch1* haploinsufficiency in humans. These different findings can be attributed to the different experimental setup and to comparing a heterozygous situation to a promoter-induced knockout (Mundy and Yang, 2008).

A recent study in zebrafish (Hammond and Schulte-Merker, 2009) could contribute to a better understanding. The authors made use of a number of zebrafish mutants in the Hh pathway (*ihha*, *ptc1*, *ptc2*, *dre* (*suppressor of fused*)), which in contrast to mice are viable to a stage that allows investigation of bone development. Furthermore, drugs were employed acting on *smoothened*, a key mediator in the Hh pathway, to either activate or suppress Hh signaling. Using this experimental setup in combination with an *osterix*-reporter line, the authors could show that there are two populations of osteoblasts in zebrafish. One of them, at the edge of the cartilage scaffold of forming bone elements, requires Hh signaling (*ihha*), but is not sensitive to high levels of Hh signaling. A second population of osteoblasts arises within the cartilage template, where cells that have a chondroblast morphology and express *collagen2* start to express *osterix* and contribute to mineralization in situations where Hh signaling is increased. Importantly the latter population could be observed in mutants as well as on titrating *smoothened* activity with small compounds. Furthermore, the authors showed that increased Hh signaling such as in *ptc2* mutants leads to an earlier onset of osteoclast activity in developing embryos, whereas reduction of Hh signaling (*ihha*) leads to a delay in comparison to wild-type embryos. This, however, seemed to be independent of the number of surrounding osteoblasts or *ranks* expression.

In conclusion, the study could independently and *in vivo* confirm the findings of both groups: increased Hh signaling promotes differentiation of osteoblasts as well

as osteoclasts. However, in concordance with Ohba *et al.* (2008), a net increase in mineralization could be observed in zebrafish. Furthermore, this study also nicely demonstrates the functional conservation of an important pathway in skeletal homeostasis and development between teleosts and mammals.

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## II. The Evolution of Skeletal Tissues

The skeleton consists of two major subunits that evolved to a large degree independently: the dermal skeleton and the endoskeleton (Smith and Hall, 1990). The basic unit of the ancestral dermal skeleton is the odontode (Huysseune and Sire, 1998; Reif, 2006). Odontodes were already composed from bone, dentin, and a hypermineralized layer. Their development requires epithelial–mesenchymal interaction. According to Huysseune *et al.* (2009, 2010), teeth are homologous to odontodes and evolved when competent ectoderm migrated via the mouth and via the gill slits into the mouth cavity. Extant chondrichthyans (sharks and rays) retain odontode-like placoid scales in their dermal skeleton (Reif, 1982) and serve as examples for illustrating the homology between teeth and odontodes (Hall and Witten, 2007; Huysseune and Sire, 1998). Reviewing properties and modes of development of skeletal tissues in extant and extinct taxa, Hall and Witten (2007) conclude that skeletal tissues reflect the early evolution of highly plastic skeletogenic cells that can modulate their behavior in response to intrinsic and environmental signals.

The first jawless vertebrates had no vertebral bodies. Their skeleton was an odontode-based mineralized dermal skeleton; the vertebral column was only represented by the notochord (Donoghue *et al.*, 2006; Hall and Witten, 2007). An endoskeleton made from “true” collagen type 2–based cartilage evolved only after the dermal skeleton (Cole and Hall, 2004; Hall and Witten, 2007). Compared to their common ancestors (basal osteichthyans), the postcranial dermal skeleton has been completely lost in mammals and has been largely reduced in teleost fish. Scales and scale-derived fin rays of teleost fish mainly represent a reduced dentin part of the ancestral odontodes (Sire and Akimenko, 2004).

Given that all basic types of skeletal tissues were already present in early vertebrates (Hall and Witten, 2007), the characters of skeletal tissues are conserved among vertebrates (Witten and Huysseune, 2009). Consequently also transcription factors and signaling molecules that facilitate skeletal cell differentiation, and hormones that regulate the skeletal development, are conserved. Still, differences between the teleost and the mammalian skeleton exist, differences that are significant if teleosts like zebrafish or medaka are used as models in biomedical research. Teleosts evolved into the most successful group of all vertebrates with about 30,000 species. They dominate the aquatic habitats, by far the largest biosphere on the planet. In view of their enormous radiation, it would be false to assume that the mammalian skeleton is advanced and the teleost skeleton is primitive (Metscher and Ahlberg, 1999; Witten and Huysseune, 2009). In fact, many characters of the teleost

skeleton are more advanced and/or elaborated compared to mammals. One example is the teleost skull that contains twice the number of skeletal elements compared to the mammalian skull (Owen, 1845). Differences between the teleost skeleton and the mammalian skeleton may also relate to adaptations to different habitats (aquatic and terrestrial), the truncation of developmental process, and size differences (Witten and Huysseune, 2009). The following section describes similarities and differences between the mammalian and the teleost skeleton.

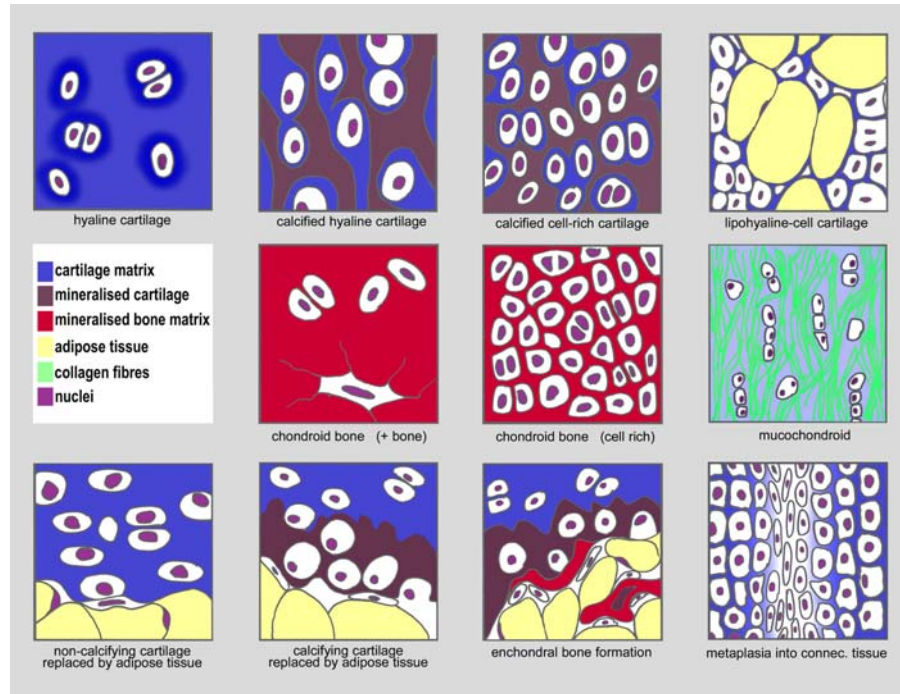
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### III. Cartilage and Bone in Teleost Fish

The major categories of skeletal tissues (cartilage, bone, dentine, and enamel/enameloid) and the major categories of skeletal cells (chondroblasts, chondrocytes, osteoblasts, bone lining cells, osteocytes, osteoclasts, odontoblasts, ameloblasts) are present in both teleosts and mammals (Huysseune, 2000; Witten and Huysseune, 2009). Apart from dentine, enamel/enameloid, and bone of attachment, which are restricted to the dermal skeleton, all skeletal tissues and respective cells occur in the teleost dermal and endoskeleton. The first step in the development of “regular” cartilage in teleosts is the condensation of mesenchymal cells (blastema stage) that develop into closely packed prechondroblasts (Huysseune and Sire, 1992a). These cells differentiate into chondroblasts and finally become separated through the secretion of extracellular cartilage matrix. Perichondral bone formation is the basic process of ossification of the cartilaginous preformed teleost endoskeleton. Different from mammals, it is often not linked to endochondral bone formation (Hall, 1998; Huysseune, 2000; Witten and Villwock, 1997) (Fig. 1). Perichondral bone is laid down at the surface of the cartilaginous template by cells that were formerly part of the perichondrium. The cells have now characteristics of osteoblasts and secrete bone matrix or a mixture of cartilage and bone matrix (Huysseune, 2000; Huysseune and Sire, 1992a; Verreijdt *et al.*, 2002). On the beginning of perichondral bone formation, the former perichondrium has become a periosteum and further thickening of the bone is carried out through deposition of bone by the osteogenic cells. A typical element of the teleost endoskeleton consists of a persisting cartilage rod inside a bone tube with cartilage sticking out as a condyle. This applies especially to smaller teleost species, such as medaka and zebrafish where endochondral bone formation is uncommon (but does occur). Cartilage remains inside the bone shaft and if cartilage is removed, it is replaced by adipose tissue (Witten *et al.*, 2001; Witten *et al.*, 2010) (Fig. 1). Replacement of cartilage by spongiosa (endochondral bone formation) can more readily be observed in larger teleost species such as carp (*Cyprinus carpio*) and salmon (*Salmo salar*).

Bone formation in teleosts can be intramembranous, perichondral, or endochondral. In the endoskeleton membranous apolamellae can form from perichondral bone, a process that resembles intramembranous bone formation (Witten and Huysseune, 2007). The cells involved in bone formation are osteoblasts. They derive



**Fig. 1** Hyaline cartilage and different types of tissues that are intermediate between bone and cartilage and between cartilage and connective tissues, which typically occur in teleosts (rows 1 and 2). Different modes of cartilage replacement that typically occur in teleosts (row 3). See Witten *et al.* (2010) for a complete list of cartilage tissues. (See color plate.)

from osteoprogenitor cells, the mesenchymal source of which has not been unequivocally identified in fish. Osteoblasts display various morphologies, depending on their secretory activity and on their position on the bone: they can be pear-shaped, spindle-shaped, or cuboidal with a pseudoepithelial arrangement (Huyseune, 2000; Witten and Hall, 2002). Secretory osteoblasts have a polarized appearance, with a highly basophilic cytoplasm indicative of intensive protein production. Osteoblasts in acellular bone (see definition below) show a polarized secretion of bone matrix, continuously withdraw from the surface, and are thus never incorporated into the matrix (Ekanayake and Hall, 1987, 1988; Huyseune, 2000; Meunier, 1983; Weiss and Watabe, 1979). Structurally, bone tissue in teleost fish develops first as woven bone. Subsequently, parallel-fibered and lamellar bone develops in more mature individuals. In larger individuals lamellar bone can also form osteons (Meunier, 2002; Moss, 1961a; Smith-Vaniz *et al.*, 1995; Witten and Hall, 2002, 2003). As fish have no hematopoietic tissue inside the bone marrow, bone marrow spaces are filled with fat tissue, besides nerves and blood vessels and some connective tissue cells (Huyseune, 2000; Witten *et al.*, 2001).



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#### IV. Intermediate Skeletal Tissues

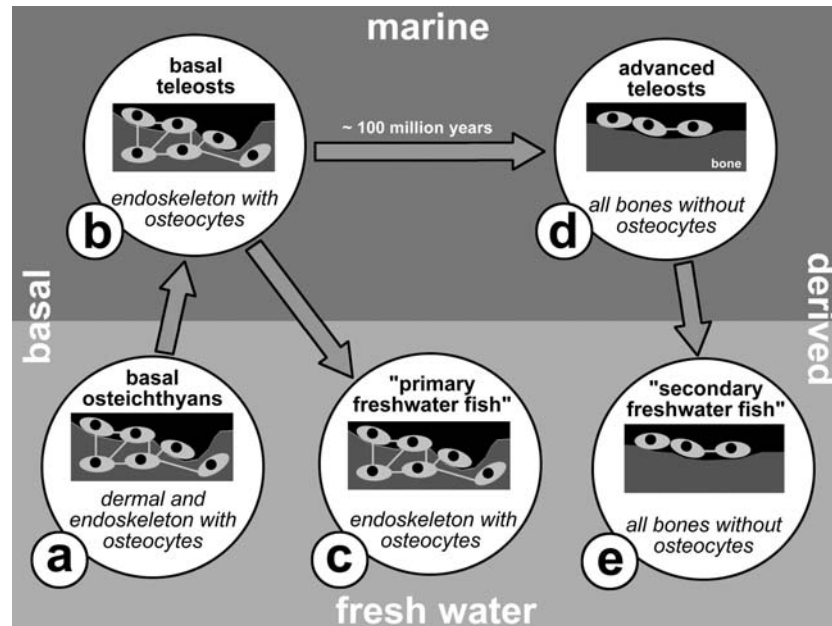
Compared to mammals, additional skeletal tissue subtypes are recognized in teleost fish as part of the regular (nonpathological, nonregenerating) skeleton (Benjamin, 1988, 1990; Benjamin *et al.*, 1992; Hall and Witten, 2007; Huysseune, 2000; Meunier and Huysseune, 1992; Witten and Huysseune, 2007) (Fig. 1). Benjamin (1990) describes seven categories of cartilage: (a) hyaline cell cartilage, (b) zellknorpel, (c) fibro/cell-rich cartilage, (d) elastic/cell-rich cartilage, (e) cell-rich hyaline cartilage, (f) matrix-rich hyaline cartilage, and (g) scleral cartilage. Secondary cartilage and chondroid cartilaginous tissues also develop on cranial dermal bones (Benjamin, 1989; Beresford, 1993; Gillis *et al.*, 2006; Huysseune, 2000; Witten and Hall, 2002). The best studied teleost “intermediate skeletal” tissue is chondroid bone (Fig. 1). Chondroid bone exhibits characteristics of bone and of cartilage and develops from osteogenic precursors (Huysseune, 1986; Huysseune and Verraes, 1986). This tissue contains chondrocyte-like cells (devoid of cell processes) surrounded by a bone-like matrix (Beresford, 1981; Huysseune and Sire, 1990; Huysseune and Verraes, 1990; Witten and Hall, 2002). Chondroid bone occurs in basal teleosts with osteocyte-containing bone and in advanced teleosts with acellular bone, and must not be confused with cellular bone (Beresford, 1981, 1993; Gillis *et al.*, 2006; Huysseune and Sire, 1990; Huysseune and Verraes, 1990; Meunier and Huysseune, 1992; Witten and Hall, 2002). Chondroid bone can also be remodeled into lamellar bone (Gillis *et al.*, 2006; Witten and Hall, 2002, 2003).

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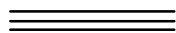
#### V. Osteocyte-Containing Bone and Acellular Bone

Like mammalian bone, the bone of more basal teleosts such as zebrafish contains osteocytes (Fig. 2). The density of osteocytes in teleost bone can vary considerably, from one species to another and within the skeleton of one species (Moss, 1961b). No data about the number of osteocytes in teleost bone are available, but in mammals, osteocytes represent 95% of all bone cells and cover 96% of all bone surfaces (Franz-Odendaal *et al.*, 2006; Witten and Huysseune, 2010). In contrast, advanced teleosts such as medaka possess acellular or anosteocytic bone, that is, bone that has no enclosed osteocytes (Ekanayake and Hall, 1987; Kölliker, 1859; Meunier and Huysseune, 1992; Moss, 1961b; Witten *et al.*, 2004) (Fig. 2). With few exceptions, the presence or absence of osteocytes is uniform in all elements of the teleost skeleton (for exceptions, see Meunier, 1989; Moss, 1961a). More basal teleosts such as salmonids and cyprinids (the group to which zebrafish belong), with cellular bone in the endoskeleton, have scales and fin rays that are acellular (Meunier, 1989). Different from dentine, no cell processes penetrate acellular bone. This bone bears however resemblance to atubular dentine that typifies the first-generation teeth of teleosts and to acellular mammalian cementum, both of which are also acellular and are not penetrated by cell processes (Ekanayake and Hall, 1987, 1988; Franz-



**Fig. 2** Relationships between phylogeny, environment, the presence of osteocytes, and the type of osteoclasts in teleost fish such as zebrafish (c) and medaka (e). Basal osteichthyans, which also gave rise to tetrapods, and basal teleosts (a and b) have bone that contains osteocytes, similar to mammalian bone. These fish have mononucleated and many multinucleated osteoclasts. Osteocytes and multinucleated osteoclasts have been preserved during a “first” wave of freshwater reinvasion by teleost fish (e; “primary freshwater fish” such as zebrafish). During a long evolutionary period in the marine environment, osteocytes disappeared (acellular bone) in almost all advanced teleosts (d). The typical osteoclast type of advanced teleosts is mononucleated. This character was maintained when advanced teleosts invaded the fresh water (e; “secondary freshwater fish,” e.g., medaka). Thus, teleosts that live in fresh water (or in the marine environment) can have different bone types and different types of bone resorbing cells. Modified after Witten and Huyssseune (2009).

Odendaal *et al.*, 2006; Meunier, 1989; Sire *et al.*, 2002; Weiss and Watabe, 1979). The formation of acellular bone resembles dentine formation. A polarized secretion of bone matrix ensures that cells never become entrapped in the bone matrix (Huyssseune, 2000).



## VI. Development of Teleost Vertebral Bodies, A Derived Process

An acellular mineralized tissue particular to all teleosts is the mineralized notochord sheath (Arratia *et al.*, 2001; Bensimon-Brito *et al.*, 2010; Grotmol *et al.*, 2003; Inohaya *et al.*, 2007; Nordvik *et al.*, 2005). Unlike other vertebrates, formation of teleost vertebral bodies does not start with a cartilaginous anlage and

also early bone is lacking. In teleosts, vertebral body development starts with the mineralization of the notochord sheath. The notochord sheath consists of a cartilage-like matrix, rich in proteoglycans and collagen type II, covered by a thin layer of elastin. There is an ongoing debate whether sclerotomal-derived cells from outside or notochord cells from inside facilitate notochord sheath mineralization. Increasing evidence suggests that notochord cells facilitate mineralization of the vertebral body anlagen (Bensimion-Brito *et al.*, 2010; Nordvik *et al.*, 2005). Only the second phase of vertebral body development involves the apposition of bone onto the mineralized notochord sheath, similar to the intramembranous bone formation (Ekanayake and Hall, 1988; Hall and Ekanayake, 1991; Grotmol *et al.*, 2003; Nordvik *et al.*, 2005). Although no cartilage contributes to the initial formation of teleost vertebral bodies (Nordvik *et al.*, 2005; Witten and Villwock, 1997), in larger individuals cartilage at the base of the arches undergoes endochondral ossification and bone that derives from this process becomes part of the vertebral body (Zylberberg and Meunier, 2008). Mineralization of the notochord sheath and the lack of cartilaginous anlagen are derived characters since basal bony fish have, similar to tetrapods (including mammals), cartilaginous vertebral body precursors (Arratia, 1983).

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## VII. Remodeling of the Teleost Skeleton

The absence of osteocytes in advanced teleosts raises questions about the regulation of bone remodeling, since cell processes from osteocytes and odontoblasts function as stress sensors in other systems. Osteocytes are believed to govern bone remodeling in response to mechanical load (Burger *et al.*, 1995; Burger *et al.*, 2003). Estimates about the percentage of bone that is resorbed and replaced by new bone in humans range between 4 and 10% per year (Delling and Vogel, 1992; Manolagas, 2000). Such estimates are lacking for teleost fish but a regular resorption and rebuilding of scales and bony skeletal elements is well documented for Atlantic salmon (Kacem *et al.*, 1998; Persson *et al.*, 2000; Witten and Hall, 2002, 2003). While we can expect that the cellular composition of the teleost skeleton affects if and how bone is remodeled, a number of additional factors affect the process. The main characteristics that distinguish teleosts from mammals with respect to skeletal remodeling are as follows (reviewed by Witten and Huysseune, 2009):

- (1) In mammals, bone resorbing cells (osteoclasts) originate from hematopoietic tissue located in the bone marrow. The hematopoietic tissue also releases factors that regulate the respective activities of osteoclasts and osteoblasts. Such an intimate spatial relationship between bone resorbing cells and hematopoietic cells does not exist in teleosts as most bone marrow spaces are filled with adipose tissue and hematopoiesis takes place in the head kidney (Field *et al.*, 1995; Song *et al.*, 2004; Witten *et al.*, 2001).

- (2) The lack of osteocytes in advanced teleosts coincides with an altered morphology of bone resorbing cells and an alternative mode of bone resorption. Osteoclasts of advanced teleosts are predominately small, mononucleated cells that can perform resorption without generating typical resorption lacunae (Kemp, 2003; Weiss and Watabe, 1979; Witten, 1997; Witten and Huysseune, 2010).
- (3) In mammals, endochondral bone formation is a prime cause of skeletal resorption and remodeling. Typical endochondral ossification is, however, often lacking in teleosts, especially in species with small individuals such as medaka and zebrafish. In addition, in all teleosts, vertebral bodies develop (ossify) without cartilaginous precursors and thus initially without remodeling (Arratia *et al.*, 2001; Bensimon-Brito *et al.*, 2010; Grotmol *et al.*, 2003; Inohaya *et al.*, 2007; Nordvik *et al.*, 2005).
- (4) Regulation of plasma calcium content is crucial for all terrestrial vertebrates and the skeleton is tightly integrated into the animals' calcium homeostasis. Teleosts have a different approach. They obtain and release calcium from and into the water via their gills and the skeleton is not used as a source or deposit of calcium (Guerreiro *et al.*, 2002; Lall and Lewis-McCrea, 2010; Perry *et al.*, 2003). In particular, resorption of the endoskeleton may be used only as a last mineral resort under extreme conditions (Moss, 1962; Takagi and Yamada, 1991, 1992). When there is a need for skeletal resorption, minerals are first mobilized from the postcranial dermal skeleton (scales) and therefore minerals must not be released from the endoskeleton (Persson *et al.*, 1998; Persson *et al.*, 1999; Persson *et al.*, 2000).
- (5) The acellular teleost skeleton responds and adapts to mechanical load (Huysseune *et al.*, 1994; Kranenbarg *et al.*, 2005; Meyer, 1987; Witten *et al.*, 2005a) but the lack of osteocytes in advanced teleosts implies that bone remodeling in response to mechanical load must be triggered by other cell types (Witten and Huysseune, 2009).
- (6) Different from sharks, teleosts are capable of repairing their endoskeleton (Ashhurst, 2004; Clement *et al.*, 1992; Moss, 1962, 1977) but the regenerative capacity of elements of the teleost dermal skeleton (fin rays and scales) largely exceeds the regenerative capacity of the endoskeleton (Akimenko and Smith, 2007; Huysseune *et al.*, 2009).
- (7) Teleosts replace their teeth throughout life, a process that requires resorption of teeth, tooth attachment bone, and dentigerous bone (Huysseune, 1983, 2000; Huysseune and Sire, 1992b; Huysseune and Witten, 2008; Witten and Huysseune, 2010; Peyer, 1968; Witten *et al.*, 2005b).
- (8) Teleosts never stop growing, and certain skeletal elements develop rather late. Thus, growth-related skeletal modeling continues throughout life and should not be mistaken for metabolism-related skeletal remodeling (Kacem *et al.*, 1998; Meunier, 2002; Persson *et al.*, 1998, 1999; Reznick *et al.*, 2002; Smith-Vaniz *et al.*, 1995; Witten and Hall, 2002, 2003).

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## VIII. Conclusions

The aim of this chapter is to highlight similarities and differences of skeleton formation among teleosts, and between teleosts and mammals. Through the course of evolution different forms of bone have evolved, but all the basic types of skeletal tissues have already been present in ancestral jawless vertebrates. What might appear as significant differences today (e.g., osteocyte-containing bone vs. acellular bone and mononucleated vs. multinucleated osteoclasts) are a variation of a common scheme. Not surprisingly, there is mounting evidence that the genetic control of osteogenesis is conserved and shared among all vertebrates. In other areas, most notable vascular and endothelial cell biology, the close interaction of researchers using the full array of methodologies (from *in vitro* cell culturing systems, morpholino knockdown studies, *in vivo* imaging of cell behavior in zebrafish embryos, inducible knockouts in mice, antibody staining on histological human material) has helped significantly to advance the field as a whole. Comparable progress can be made in the area of osteogenesis if resources are used in a complementing manner.

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