CHAPTER

8

Zebrafish Integumentary System

Andrew J. Aman[1], *David M. Parichy*[1,2]

[1]Department of Biology, University of Virginia, Charlottesville, VA, United States of America; [2]Department of Cell Biology, University of Virginia, Charlottesville, VA, United States of America

Introduction

The zebrafish, like all animals, is covered with sophisticated skin that acts as both a wall and a window to the external environment. The skin is traditionally divided into three layers. A superficial epidermis—comprising a stratified epithelium—provides barrier and sensory function. The underlying dermis is dominated by bony elasmoid scales and a deep collagenous stroma that together armor and support the muscles and organs. The relatively thin hypodermis lies deeper, at the boundary between skin and muscle, and harbors adipocytes and pigment cells. Below, we give a brief primer on the development and anatomy of zebrafish skin.

Epidermal Development and Anatomy

The epidermis represents the ultimate boundary between the fish and its environment. It forms a semiimpermeable barrier while also allowing sensory functions. The primary epidermis, known as the enveloping layer, first develops before gastrulation (Kimmel, Warga, & Schilling, 1990). Following gastrulation, the enveloping layer becomes the outermost skin cell layer, the periderm, and basal cells arise from nonneural ectoderm, thereby generating a bilayered epidermis (Bakkers, Hild, Kramer, Furutani-Seiki, & Hammerschmidt, 2002; Lee & Kimelman, 2002). Periderm cells are connected by tight junctions forming a solute barrier and are decorated with elaborate, wavy microridges that increase epidermal surface area and likely aid in mucous retention (Hawkes, 1974; Kiener, Selptsova-Friedrich, & Hunziker, 2008; Lam, Mangos, Green, Reiser, & Huttenlocher, 2015; Whitear 1970). This bilayered state persists through the early larval period until 6–7 standardized standard length [SSL (Parichy et al. 2009); ~6–7 mm standard length], when descendants of basal cells generate the intermediate suprabasal cell layers (Guzman, Ramos-Balderas, Carrillo-Rosas, & Maldonado, 2013; Lee, Asharani, & Carney, 2014).

In adult skin, there are two to eight layers of suprabasal cells over the surface of the fish, whereas superficial periderm and deep basal cells remain as monolayers (Fig. 8.1). Similar to mammalian epidermis, basal cells serve as epidermal stem cells, and their proliferation as well as suprabasal cell proliferation is regulated by ΔN-p63. Intermediate suprabasal cells are the most proliferative cells in the epidermis and most appear undifferentiated. It is possible they serve as a transient amplifying population, though precise lineages and stem cell kinetics of the epidermis remain unresolved (Guzman et al. 2013; Le Guellec, Morvan-Dubois, & Sire, 2004; Lee & Kimelman, 2002; Quilhac and Sire 1999; Richardson et al. 2013).

The epidermis harbors several additional cell types. Goblet cells secrete mucous and club cells produce alarm substances (Jevtov, Samuelsson, Yao, Amsterdam, & Ribbeck, 2014). Ionocytes and chemosensory cells aid in maintaining chemical homeostasis (Coccimiglio & Jonz, 2012; Cruz, Chao, & Hwang, 2013; Hwang & Chou, 2013). Somatosensory cells send their nerve endings into the epidermis enabling a sense of touch (Rasmussen, Vo, Sagasti, 2018). Moreover, the mechanosensory neuromasts of the lateral line are hosted by the epidermis and enable detection of water motions against the surface of the fish (Lee, Asharani, Carney, 2014; Metcalfe, 1985).

https://doi.org/10.1016/B978-0-12-812431-4.00008-7

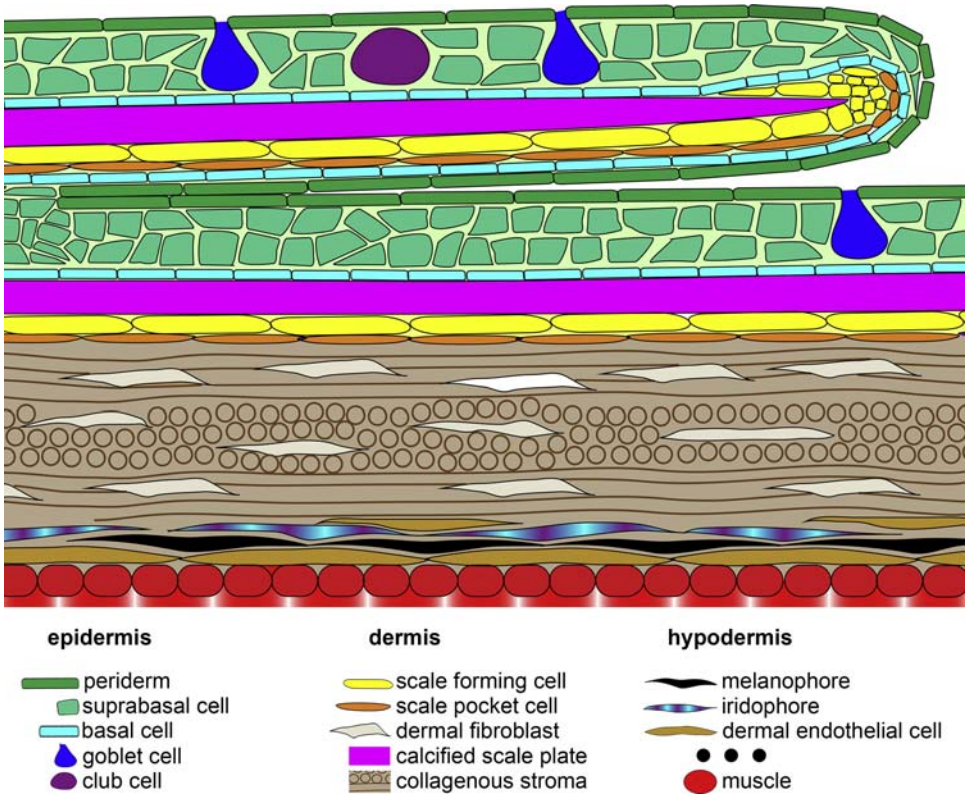


FIGURE 8.1 **Schematic section through adult zebrafish skin.** Depicted is a simplified coronal skin section at the level of a dark stripe in the trunk. For clarity, adipocytes, blood vessels, nerves, chemosensory cells, ionocytes, immune cells, and other cells are omitted, and those cell types shown are rendered with exaggerated thickness. The outermost periderm (green) and suprabasal cells (teal) overlie the basal cell monolayer (cyan). Epidermis wraps around the posterior margin of the calcified scale plate (magenta) and the scale-forming cells (yellow) and is closely associated with the scale pocket cells (orange). The epidermis contains specialized cell types including goblet cells (blue) and club cells (purple), secreting mucous and alarm substance, respectively. The surface of the fish is protected by at least two scales. Beneath the scales lies a collagenous stroma, the stratum compactum (tan), harboring dermal fibroblasts (beige). Dermal endothelial cells of the hypodermis (light brown) line the muscles (red) at the deep limit of the skin. Pigment cells, including melanophores (black) and iridophores (cyan/purple), reside in close proximity to hypodermal cells.

Development and Anatomy of the Hypodermis and Collagenous Dermal Stroma

The dermis contains a dense collagenous stroma—the stratum compactum—that imbues the skin with mechanical strength. Basal epidermal cells begin producing a primary collagenous stroma by 24 h postfertilization (hpf). Once initiated, the stroma thickens throughout the life of the fish. By 72 hpf, thin layer of dermal endothelial cells begins to accumulate along the surface of the muscles to constitute the hypodermis. These cells also contribute to the growth of the primary stroma. Basal epidermal and hypodermal cells continue to produce collagen through the early larval period, building an ever thicker collagenous stroma. Hypodermal cells remain a sparse layer in adult fish, where they likely provide trophic and other support to pigment cells comprising the stripe pattern and to dermal adipocytes (Fig. 8.1) (Hirata, Nakamura, & Kondo, 2005; Lang, Patterson, Gordon, Johnson, & Parichy, 2009; Le Guellec, Morvan-Dubois, Sire 2004; Minchin and Rawls 2017).

Coincident with stratification of the epidermis, the primary collagenous stroma of the dermis becomes organized into a plywood-like arrangement of orthogonally aligned collagen fibers. This stroma remains devoid of cells until ~8 mm SSL (Parichy, Elizondo, Mills, Gordon, Engeszer, 2009) when it is populated by dermal fibroblasts to generate the stratum compactum, a tough yet transparent structural component of the skin that resists tearing and may provide elastic recoil to the swim stroke (Fig. 8.1) (Szewciw and Barthelat 2017).

Development and Anatomy of Elasmoid Scales

Hundreds of arrowhead-shaped, calcified scales are embedded in the dermis of adult zebrafish (Fig. 8.2A). These overlap precisely like roof tiles so that every position along the body is covered by at least two scales, providing flexible armor that likely protects against puncture injuries that might be inflicted by cooccurring

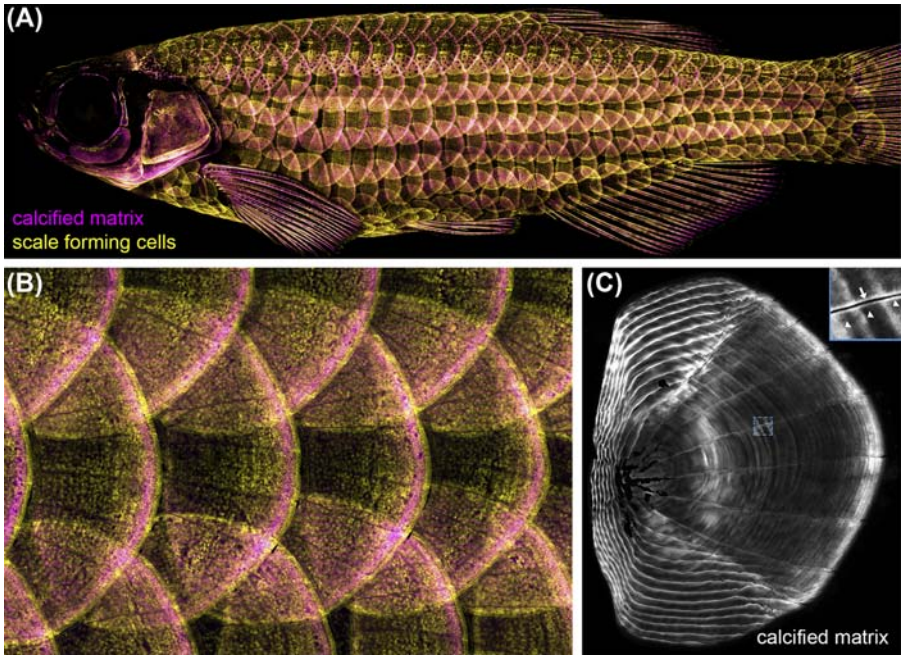


FIGURE 8.2 Distribution and morphology of zebrafish scales. (A) Scales cover the postcranial surface of the fish, here revealed by expression of *sp7:EGFP* in scale-forming cells (yellow) as well as Alizarin Red-S labeled calcified matrix (magenta). (B) Overlapping scales are arranged in a half-offset, hexagonal grid. (C) A freshly plucked, Alizarin Red-S stained scale shows a relatively heavily calcified limiting layer at the posterior margin and concentric circuli (arrowheads in inset). Note the different arrangement of circuli in the protruding posterior and embedded anterior portion of the scale. The protruding portion of the scale contains matrix-free radii that organize and house neurons and vasculature of the skin (arrow in inset).

predators or other types of trauma (Figs. 8.1, 8.2A,B) (Engeszer, Patterson, Rao, & Parichy, 2007; Sire, Allizard, Babiar, Bourguignon, Quilhac, 1997; Zhu, Szewciw, Vernerey, Barthelat, 2013).

After the stratum compactum forms, in 8–10 SSL larvae, dermal cells accumulate at the superficial limit of the dermis, in close proximity to the epidermis. These superficial cells aggregate to form scale papillae (Le Guellec et al. 2004; Mongera and Nüsslein-Volhard 2013; Shimada et al. 2013; Sire et al. 1997). Dermal papillae form first in the caudal peduncle and above the ribs. Additional papillae are added in rows and columns to generate a half-offset hexagonal grid of scales (Fig. 8.2A,B) (Aman, Fulbright, & Parichy, 2018; Sire et al. 1997).

Initiation of scale papillae relies on interactions between epidermis and dermis involving ectodysplasin, Wnt/β-catenin, and fibroblast growth factor (Fgf) signaling pathways (Aman et al. 2018; Daane, Rohner, Konstantinidis, Djuranovic, & Harris, 2015; Harris et al. 2008; Rohner et al. 2009). Remarkably, interactions between signaling pathways and the cell behaviors they govern during zebrafish scale development resemble those governing formation and patterning of skin appendage like feathers and hair in terrestrial vertebrates. This implies that, despite profound differences in matrix composition—dermal calcified matrix of zebrafish scales and epidermal keratin of terrestrial appendages—all skin appendages likely share a common ancestry, and mechanisms that govern their early development are similar across vertebrates.

Following aggregation, dermal papillae produce the initial calcified matrix of the scale plate (Aman, Fulbright, Parichy, 2018; Sire et al. 1997). Developing scales are oriented toward the posterior by the planar cell polarity machinery, and their growth is driven by Sonic hedgehog (Shh) and Fgf-dependent proliferation and growth of osteoblast-like scale-forming cells accompanied by expansion of the calcified matrix (Aman et al. 2018; Cox et al. 2018; Iwasaki, Kuroda, Kawakami, & Wada, 2018; Rasmussen et al. 2018; Sire et al. 1997).

The structure of the scale plate consists of a basal layer of weakly ossified collagen, called isopedine, capped by the more strongly ossified external layer. At the posterior margin of the scale, a highly calcified, collagen-poor limiting layer is present (Fig. 8.2C). The scale-forming cells line the deep aspect of the scale and loop around the limiting layer at the posterior scale margin (Fig. 8.1). Gene expression in these posterior margin scale-forming cells is distinct from other scale-forming cells. It is this population that proliferates to add scale-forming cells during growth (Aman et al. 2018; Cox et al. 2018; Iwasaki et al. 2018). As growth proceeds, limiting layer matrix is deposited in waves, leading to concentric arcs of more heavily calcified circuli in the mature scale (Fig. 8.2C) (Sire et al. 1997). The scale

plate is also punctuated by matrix-free channels, the radii, that organize and house neuronal processes and vasculature of the skin (Rasmussen et al. 2018; Sire et al. 1997). During scale outgrowth, epidermal Shh triggers accumulation of a thin layer of scale pocket cells underneath the scale-forming cells (Fig. 8.1). Epidermal cells invaginate along these cells yielding the final partially protruding organization of the adult scale (Aman et al. 2018; Sire et al. 1997).

The evolutionary relationships between elasmoid scale extracellular matrix and calcified matrices in mammals—enamel, dentin, cartilage, and bone—remain uncertain. The precise homologies of cells secreting these matrices are similarly ambiguous. Paleontological and other evidence indicate that thin, flexible scales of zebrafish and other teleosts descended from heavy, enameled rhomboid scales of ancient vertebrates. Such rhomboid scales contain clear examples of matrix resembling enamel, dentine, and bone, a state preserved in teleost sister groups, the elasmobranchs (sharks/rays), polypterids (bichirs/reedfish), and holosteans (bowfin/gars) (Janvier, 1996; Märss, 2006; Sire 1990; Sire and Huysseune 2003). In this respect, scales of zebrafish resemble mammalian teeth perhaps more than mammalian bone.

The cells that produce the ossified matrix of the zebrafish scale plate have been called osteoblasts because the matrix they secrete is calcified like bone and because they express *sp7/osterix*, encoding a transcription factor utilized by mammalian osteoblasts (Cox et al. 2018; Iwasaki et al. 2018; Metz, de Vrieze, Lock, Schulten, & Flik, 2012; Rasmussen et al. 2018). It is important to note, however, that mammalian *sp7* is a general regulator of multiple cell types that produce calcified matrices, including enamel-producing ameloblasts and dentin-expressing odontoblasts (Bae et al. 2018).

To acknowledge the presently ambiguous homology of zebrafish-calcified matrix-producing cells, and to honor the prior nomenclatural recommendations of Jean-Yves Sire, we recommend referring to these Sp7+ cells simply as scale-forming cells, rather than osteoblasts per se (Sire et al. 1997). Deeper investigation of gene expression and its regulatory network should allow a more precise understanding of scale-forming cell evolution and will enable rigorous comparisons of biology between these and other matrix-secreting populations across vertebrates.

Zebrafish Skin as a Model for Skin Disease, Wound Healing and Regeneration

The optical transparency and superficial location of zebrafish skin make it exceptionally accessible to manipulation and imaging. These advantages, coupled with histological and molecular similarities to human skin, make zebrafish an attractive system for understanding cellular and molecular basis of skin disease, wound healing, and regeneration. Indeed, zebrafish with mutations in genes associated with human cutaneous disease often recapitulate aspects of these diseases and can provide insights into pathology (Feitosa, Richardson, Bloch, & Hammerschmidt, 2011; Hatzold et al. 2016; Li et al. 2011a, 2011b). Zebrafish skin is also highly regenerative and is proving to be an excellent system for uncovering mechanisms that underlie injury response, wound healing, and tissue regeneration (Armstrong, Henner, Stewart, & Stankunas, 2017; Chen et al. 2016; Cox et al. 2018; Gault, Enyedi, & Niethammer, 2014; Kang, Nachtrab, & Poss, 2013; Richardson et al. 2013, 2016).

Outlook

Zebrafish skin affords the biomedical research community a powerful system to address fundamental questions of stem cell biology, patterning, morphogenesis, regeneration, and disease. Emerging genetic reagents, as well as imaging and analytical methods, will further enable the study of zebrafish skin and will likely contribute novel insights into these important areas of biology and pathology.

Acknowledgments

A.A. is supported by NIH R35 GM122471 to D.M.P.

References

Aman, A. J., Fulbright, A. N., & Parichy, D. M. (2018). Wnt/beta-catenin regulates an ancient signaling network during zebrafish scale development. *Elife, 7*. https://doi.org/10.7554/eLife.37001.

Armstrong, B. E., Henner, A., Stewart, S., & Stankunas, K. (2017). Shh promotes direct interactions between epidermal cells and osteoblast progenitors to shape regenerated. *Zebrafish Bone Development, 144*, 1165–1176. https://doi.org/10.1242/dev.143792.

Bae, J. M., et al. (2018). Specificity protein 7 is required for proliferation and differentiation of ameloblasts and odontoblasts. *Journal of Bone and Mineral Research, 33*, 1126–1140. https://doi.org/10.1002/jbmr.3401.

Bakkers, J., Hild, M., Kramer, C., Furutani-Seiki, M., & Hammerschmidt, M. (2002). Zebrafish DeltaNp63 is a direct target of Bmp signaling and encodes a transcriptional repressor blocking neural specification in the ventral ectoderm. *Developmental Cell, 2*, 617–627.

Chen, C. H., Puliafito, A., Cox, B. D., Primo, L., Fang, Y., Di Talia, S., et al. (2016). Multicolor cell barcoding technology for long-term surveillance of epithelial regeneration in Zebrafish. *Developmental Cell, 36*, 668–680. https://doi.org/10.1016/j.devcel.2016.02.017.

Coccimiglio, M. L., & Jonz, M. G. (2012). Serotonergic neuroepithelial cells of the skin in developing zebrafish: Morphology, innervation and oxygen-sensitive properties. *Journal of Experimental Biology, 215*, 3881–3894. https://doi.org/10.1242/jeb.074575.

Cox, B. D., De Simone, A., Tornini, V. A., Singh, S. P., Di Talia, S., & Poss, K. D. (2018). In toto imaging of dynamic osteoblast behaviors in regenerating skeletal bone. *Current Biology, 28*, 3937–3947. https://doi.org/10.1016/j.cub.2018.10.052. e3934.

Cruz, S. A., Chao, P. L., & Hwang, P. P. (2013). Cortisol promotes differentiation of epidermal ionocytes through Foxi3 transcription factors in zebrafish (*Danio rerio*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 164*, 249–257. https://doi.org/10.1016/j.cbpa.2012.09.011.

Daane, J. M., Rohner, N., Konstantinidis, P., Djuranovic, S., & Harris, M. P. (2015). *Parallelism and epistasis in skeletal evolution identified through use of phylogenomic mapping strategies molecular biology and evolution*. https://doi.org/10.1093/molbev/msv208.

Engeszer, R. E., Patterson, L. B., Rao, A. A., & Parichy, D. M. (2007). *Zebrafish in the wild: A review of natural history and new notes from the field zebrafish* (Vol. 4, pp. 21–40). https://doi.org/10.1089/zeb.2006.9997.

Feitosa, N. M., Richardson, R., Bloch, W., & Hammerschmidt, M. (2011). Basement membrane diseases in zebrafish. *Methods Cell Biology, 105*, 191–222. https://doi.org/10.1016/b978-0-12-381320-6.00008-4.

Gault, W. J., Enyedi, B., & Niethammer, P. (2014). Osmotic surveillance mediates rapid wound closure through nucleotide release. *The Journal of Cell Biology, 207*, 767–782. https://doi.org/10.1083/jcb.201408049.

Guzman, A., Ramos-Balderas, J. L., Carrillo-Rosas, S., & Maldonado, E. (2013). A stem cell proliferation burst forms new layers of P63 expressing suprabasal cells during zebrafish postembryonic epidermal development. *Biol Open, 2*, 1179–1186. https://doi.org/10.1242/bio.20136023.

Harris, M. P., Rohner, N., Schwarz, H., Perathoner, S., Konstantinidis, P., & Nüsslein-Volhard, C. (2008). Zebrafish *eda* and *edar* mutants reveal conserved and ancestral roles of Ectodysplasin signaling in vertebrates. *PLoS Genetics, 4*, e1000206. https://doi.org/10.1371/journal.pgen.1000206.

Hatzold, J., et al. (2016). Tumor suppression in basal keratinocytes via dual non-cell-autonomous functions of a Na,K-ATPase beta subunit. *Elife, 5*. https://doi.org/10.7554/eLife.14277.

Hawkes, J. W. (1974). *The structure of fish skin Cell and Tissue Research* (Vol. 149, pp. 147–158). https://doi.org/10.1007/bf00222270.

Hirata, M., Nakamura, K.-I., & Kondo, S. (2005). Pigment cell distributions in different tissues of the zebrafish, with special reference to the striped pigment pattern Developmental. *Dynamics, 234*, 293–300. https://doi.org/10.1002/dvdy.20513.

Hwang, P.-P., & Chou, M.-Y. (2013). Zebrafish as an animal model to study ion homeostasis Pflugers. *Archiv : European Journal of Physiology, 465*, 1233–1247. https://doi.org/10.1007/s00424-013-1269-1.

Iwasaki, M., Kuroda, J., Kawakami, K., & Wada, H. (2018). Epidermal regulation of bone morphogenesis through the development and regeneration of osteoblasts in the zebrafish scale. *Developmental Biology*. https://doi.org/10.1016/j.ydbio.2018.03.005.

Janvier, P. (1996). *Early vertebrates*. New York: Oxford University Press Inc.

Jevtov, I., Samuelsson, T., Yao, G., Amsterdam, A., & Ribbeck, K. (2014). Zebrafish as a model to study live mucus physiology. *Scientific Reports, 4*, 6653. https://doi.org/10.1038/srep06653. https://www.nature.com/articles/srep06653#supplementary-information.

Kang, J., Nachtrab, G., & Poss, K. D. (2013). Local Dkk1 crosstalk from breeding ornaments impedes regeneration of injured male zebrafish fins. *Developmental Cell, 27*, 19–31. https://doi.org/10.1016/j.devcel.2013.08.015.

Kiener, T. K., Selptsova-Friedrich, I., & Hunziker, W. (2008). Tjp3/zo-3 is critical for epidermal barrier function in zebrafish embryos. *Developmental Biology, 316*, 36–49. https://doi.org/10.1016/j.ydbio.2007.12.047.

Kimmel, C. B., Warga, R. M., & Schilling, T. F. (1990). *Origin and organization of the zebrafish fate map Development* (Vol. 108, pp. 581–594).

Lam, P. Y., Mangos, S., Green, J. M., Reiser, J., & Huttenlocher, A. (2015). In vivo imaging and characterization of actin microridges. *PLoS One, 10*, e0115639. https://doi.org/10.1371/journal.pone.0115639.

Lang, M. R., Patterson, L. B., Gordon, T. N., Johnson, S. L., & Parichy, D. M. (2009). Basonuclin-2 requirements for zebrafish adult pigment pattern development and female fertility PLoS. *Forest Genetics, 5*, e1000744. https://doi.org/10.1371/journal.pgen.1000744.

Le Guellec, D., Morvan-Dubois, G., & Sire, J. Y. (2004). Skin development in bony fish with particular emphasis on collagen deposition in the dermis of the zebrafish (*Danio rerio*). *International Journal of Developmental Biology, 48*, 217–231. https://doi.org/10.1387/ijdb.031768dg.

Lee, R. T., Asharani, P. V., & Carney, T. J. (2014). Basal keratinocytes contribute to all strata of the adult zebrafish epidermis. *PLoS One, 9*, e84858. https://doi.org/10.1371/journal.pone.0084858.

Lee, H., & Kimelman, D. (2002). A dominant-negative form of p63 is required for epidermal proliferation in zebrafish. *Developmental Cell, 2*, 607–616.

Li, Q., et al. (2011a). Abca12-mediated lipid transport and Snap29-dependent trafficking of lamellar granules are crucial for epidermal morphogenesis in a zebrafish model of ichthyosis. *Disease Models & Mechanism, 4*, 777–785. https://doi.org/10.1242/dmm.007146.

Li, Q., Frank, M., Thisse, C. I., Thisse, B. V., & Uitto, J. (2011b). Zebrafish: A model system to study heritable skin diseases. *Journal of Investigative Dermatology, 131*, 565–571. https://doi.org/10.1038/jid.2010.388.

Märss, T. (2006). Exoskeletal ultrasculpture of early vertebrates. *Journal of Vertebrate Paleontology, 26*, 235–252, 10.1671/0272-4634(2006)26[235:EUOEV]2.0.CO;2.

Metcalfe, W. K. (1985). Sensory neuron growth cones comigrate with posterior lateral line primordial cells in zebrafish. *Journal of Comparative Neurology, 238*, 218–224. https://doi.org/10.1002/cne.902380208.

Metz, J. R., de Vrieze, E., Lock, E.-J., Schulten, I. E., & Flik, G. (2012). Elasmoid scales of fishes as model in biomedical bone research. *Journal of Applied Ichthyology, 28*, 382–387. https://doi.org/10.1111/j.1439-0426.2012.01990.x.

Minchin, J. E. N., & Rawls, J. F. (2017). A classification system for zebrafish adipose tissues. *Disease Models & Mechanisms, 10*, 797–809. 10.1242/dmm.025759.

Mongera, A., & Nüsslein-Volhard, C. (2013). Scales of fish arise from mesoderm. *Current Biology, 23*, R338–R339. https://doi.org/10.1016/j.cub.2013.02.056.

Parichy, D. M., Elizondo, M. R., Mills, M. G., Gordon, T. N., & Engeszer, R. E. (2009). Normal table of postembryonic zebrafish development: Staging by externally visible anatomy of the living fish. *Developmental Dynamics, 238*, 2975–3015.

Quilhac, A., & Sire, J. Y. (1999). Spreading, proliferation, and differentiation of the epidermis after wounding a cichlid fish. *Hemichromis bimaculatus Anat Rec, 254*, 435–451. https://doi.org/10.1002/(sici)1097-0185(19990301)254:3<435::aid-ar15>3.0.co;2-d.

Rasmussen, J. P., Vo, N. T., & Sagasti, A. (2018). Fish scales dictate the pattern of adult skin innervation and vascularization. *Developmental Cell, 46*, 344–359. https://doi.org/10.1016/j.devcel.2018.06.019. e344.

Richardson, R., et al. (2016). *Re-epithelialization of cutaneous wounds in adult zebrafish combines mechanisms of wound closure in embryonic and adult mammals Development* (Vol. 143, pp. 2077–2088). https://doi.org/10.1242/dev.130492.

Richardson, R., Slanchev, K., Kraus, C., Knyphausen, P., Eming, S., & Hammerschmidt, M. (2013). Adult zebrafish as a model system for cutaneous wound-healing research. *Journal of Investigative Dermatology, 133*, 1655–1665. https://doi.org/10.1038/jid.2013.16.

Rohner, N., et al. (2009). Duplication of fgfr1 permits Fgf signaling to serve as a target for selection during. *Domestication Current Biology*, *19*, 1642–1647. https://doi.org/10.1016/j.cub.2009.07.065.

Shimada, A., et al. (2013). Trunk exoskeleton in teleosts is mesodermal in origin. *Nature Communications*, *4*, 1639. https://doi.org/10.1038/ncomms2643. http://www.nature.com/articles/ncomms2643#supplementary-information.

Sire, J. Y. (1990). From ganoid to elasmoid scales in the acinopterygian fishes Netherlands. *Journal of Zoology*, *40*, 75–92. https://doi.org/10.1163/156854289X00192.

Sire, J.-Y., Allizard, F., Babiar, O., Bourguignon, J., & Quilhac, A. (1997). Scale development in zebrafish (*Danio rerio*). *Journal of Anatomy*, *190*, 545–561. https://doi.org/10.1046/j.1469-7580.1997.19040545.x.

Sire, J. Y., & Huysseune, A. (2003). Formation of dermal skeletal and dental tissues in fish: A comparative and evolutionary approach. *Biological Reviews of the Cambridge Philosophical Society*, *78*, 219–249.

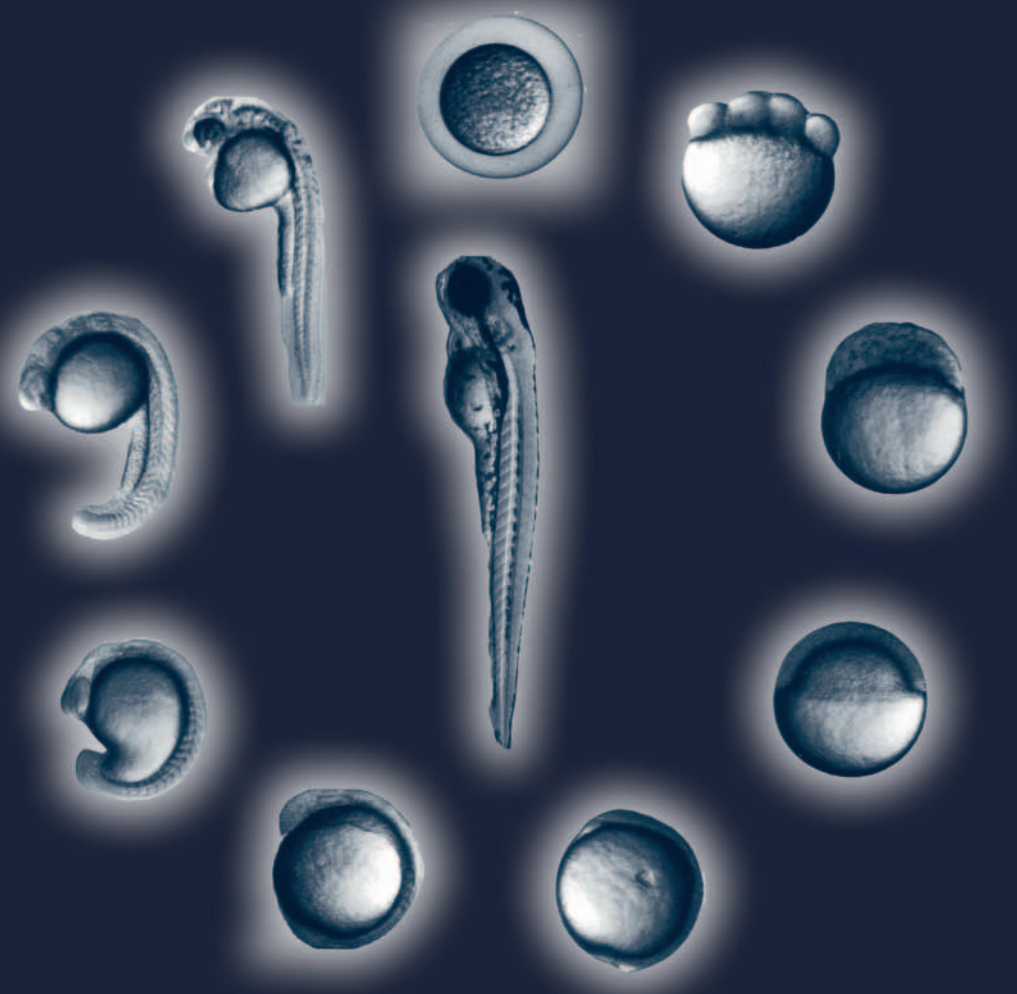
Szewciw, L., & Barthelat, F. (2017). Mechanical properties of striped bass fish skin: Evidence of an exotendon function of the stratum compactum. *Journal of the Mechanical Behavior of Biomedical Materials*, *73*, 28–37. https://doi.org/10.1016/j.jmbbm.2016.09.031.

Whitear, M. (1970). The skin surface of bony fishes. *Journal of Zoology*, *160*, 437–454. https://doi.org/10.1111/j.1469-7998.1970.tb03091.x.

Zhu, D., Szewciw, L., Vernerey, F., & Barthelat, F. (2013). Puncture resistance of the scaled skin from striped bass: Collective mechanisms and inspiration for new flexible armor designs. *Journal of the Mechanical Behavior of Biomedical Materials*, *24*, 30–40. https://doi.org/10.1016/j.jmbbm.2013.04.011.

The Zebrafish in Biomedical Research

Biology, Husbandry, Diseases, and Research Applications



EDITED BY
Samuel C. Cartner
Judith S. Eisen
Susan C. Farmer
Karen J. Guillemin
Michael L. Kent
George E. Sanders

American College of Laboratory Animal Medicine Series

THE ZEBRAFISH IN BIOMEDICAL RESEARCH

Biology, Husbandry, Diseases, and Research Applications

Edited by

Samuel C. Cartner
Animal Resources Program, University of Alabama at Birmingham
Birmingham, AL, United States of America

Judith S. Eisen
Institute of Neuroscience, University of Oregon
Eugene, OR, United States of America

Susan C. Farmer
University of Alabama at Birmingham, Birmingham, AL, United States of America

Karen J. Guillemin
Institute of Molecular Biology, University of Oregon, Eugene, OR, United States of America;
Humans and the Microbiome Program, CIFAR, Toronto, ON, Canada

Michael L. Kent
Departments of Microbiology and Biomedical Sciences, Oregon State University
Corvallis, OR, United States of America

George E. Sanders
Department of Comparative Medicine, University of Washington
Seattle, WA, United States of America

ACADEMIC PRESS
An imprint of Elsevier