CHAPTER

9

Zebrafish Pigmentation

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Introduction

The pigmentation of adult zebrafish consists of several different elements and depends on several distinct classes of pigment cells. By far the most prominent elements are the dark stripes and light "interstripes" that run horizontally across the body. Pigment cells—in ectotherms, sometimes referred to as chromatophores—are also present on the scales (conferring a darker cast dorsally than ventrally), on the head, and on the fins, where they form stripes and other arrangements.

Chromatophores are derived embryologically from the neural crest, which also contributes to the craniofacial skeleton, peripheral nervous system, and other tissues and organs (Dupin et al., 2018; Hörstadius, 1950). In zebrafish, the most abundant chromatophores are the black melanophores, yellow/orange xanthophores, and iridescent iridophores (Fujii, 1993; Johnson et al., 1995; Mort et al., 2015; Schartl et al., 2016). Melanophores are homologous to melanocytes of birds and mammals but differ from melanocytes in retaining melanin granules rather than transferring them to keratinocytes. Xanthophores contain pteridines, carotenoids, or both pigments, which are detectable by their autofluorescence. Iridophores, by contrast, depend for their iridescence on crystalline guanine, held within stacked reflecting platelets. Additional cell types—white, or white and yellow leucophores—occur in the fins (Lewis et al., 2019). As for melanophores, pigments and platelets of xanthophores, iridophores, and leucophores, are retained intracellularly. So the pigment pattern is a direct indication of chromatophore distributions.

Adult stripes consist of melanophores and sparse iridophores, whereas interstripes have densely packed iridophores and xanthophores (Fig. 9.1(Patterson and Parichy, 2009)). Such a distinctive pattern suggests behavioral or ecological significance, and indeed, pigment patterns of other fishes can have many roles: helping individuals to avoid predators, to recognize others of their own species, and to choose their mates (Marshall et al., 2018; Price et al., 2008). Laboratory studies of zebrafish suggest that pigmentation, and stripes in particular, facilitate social aggregation, or shoaling (Engeszer et al., 2008; Parichy, 2015; Rosenthal et al., 2005). However, the natural history of zebrafish remains poorly understood, and the specific functions of pigmentation have yet to be addressed in the wild.

Because stripe pattern formation is understood best, its events are described in some detail below. Other tissues are mentioned briefly, as are physiological and pathological changes that can affect pigmentation. In recent years, transgenic models of zebrafish also have been used to understand the origins and progression of melanoma and to identify potential therapies for this deadly cancer of the melanocyte lineage (Kaufman, 2016). This important but distinct topic is not reviewed here.

Stripes and Their Development

The pigmentation of adult zebrafish differs markedly from the earliest expression of this trait, in late embryos and early larvae (EL) (Fig. 9.1) (Dutton et al., 2001; Kimmel et al., 1995; Parichy et al., 2009). At these stages, melanophores occur on the head and extend posteriorly along the dorsal myotomes, wrapping around to the ventral myotomes. Melanophores also line the dorsal and ventral edges of the yolk and swim bladder. A few melanophores are found in the middle of the flank at the horizontal myoseptum. Iridophores occur sparsely in the regions occupied by melanophores. By contrast, xanthophores are scattered widely over the flank and dorsum. These cells gradually fade and are no longer apparent by ~5.0 standardized standard length (SSL, approximating 5.0 mm or ~10-day postfertilization) (McMenamin et al., 2014; Parichy et al., 2009). What, if any, function the EL pattern serves, remains unclear,

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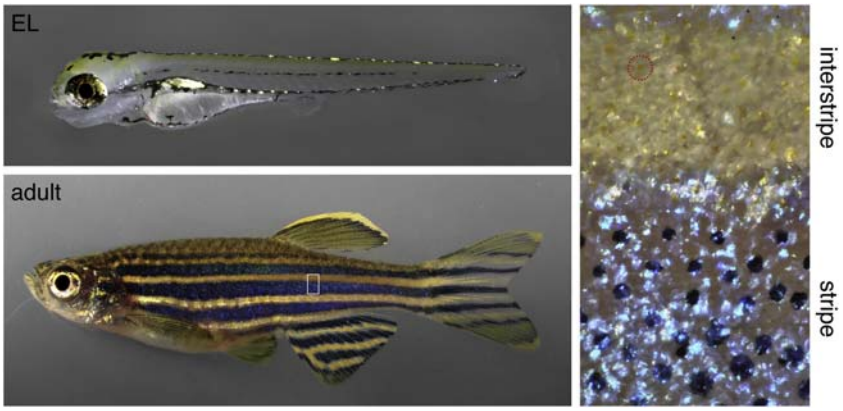

FIGURE 9.1 Embryo/early larva (EL) and adult pigmentation of zebrafish. Closeup of adult stripe and interstripe corresponds to approximate position of boxed regions. Pigment cells have been treated with epinephrine, which contracts pigment of melanophores and xanthophores toward cell centers. Melanophores are black, xanthophores appear as pale orange (e.g., red outline), and iridophores are iridescent, forming a yellowish mat in the interstripe and dispersed blue cells in the stripe.

but given the locations of melanophores and iridophores, one might imagine they help to protect stem cell populations (brain, spinal cord, gonads) or other tissues from ultraviolet light in the shallow waters where zebrafish breed (Mueller et al., 2014; Parichy, 2015).

Numerous mutants have defects in EL pigmentation and the genes corresponding to many of these have been identified (Arduini et al., 2008; Cornell et al., 2004; Elizondo et al., 2005; Kelsh et al., 1996; Odenthal et al., 1996). Often, defects arise from failures to synthesize cell-type-specific pigments or to localize them in specialized organelles (Dooley et al., 2013b; Lamason et al., 2005; Lister, 2019). Pigmentation defects in other mutants arise because of failures to specify or maintain one or more chromatophore lineages (Dutton et al., 2001; Lister et al., 1999; Lopes et al., 2008; Nord et al., 2016; Parichy et al., 2000; Petratou et al., 2018). Only a few mutants have been identified that affect the patterning or localization of chromatophores (Camargo-Sosa et al., 2019; Parichy et al., 1999; Svetic et al., 2007; Zhang et al., 2018), suggesting a robustness to these processes, or a dependence on genes having essential functions prior to EL pattern formation.

The EL arrangement of chromatophores persists with few changes until 4.5–5.0 SSL, when new chromatophores start to appear, and the adult pattern begins to form (Parichy et al., 2009) (Fig. 9.2). Although overt morphological changes are not manifest until the larva-to-adult transformation—along with other changes to the skin and other organs—remodeling of pigmentation depends on multipotent, neural-crest derived pigment cell progenitors established within the peripheral nervous system during early embryonic development (Budi et al., 2011, 2008; Dooley et al., 2013a; Hultman et al., 2009; Saunders et al., 2019; Singh et al., 2014, 2016; Tryon et al., 2011). During later adult pattern formation, some progenitors migrate to the hypodermis of the skin (Aman & Parichy, this volume) where they differentiate as iridophores that will form a "primary" interstripe in the middle of the flank. Iridophores first appear anteriorly, then far posteriorly, and then in between, until the interstripe is continuous (Parichy et al., 2009). The positioning of iridophores requires normal myotome development, as mutants with defects in myoseptal boundaries have disrupted interstripes (Frohnhofer et al., 2013; Parichy et al., 2015). Additional signals required for iridophore differentiation, proliferation, and survival come from fibroblasts or other cells of the skin, or superficial cells of the myotomes (Fadeev et al., 2018; Krauss et al., 2014; Lang et al., 2009; Mo et al., 2017; Spiewak et al., 2018).

Shortly after adult iridophores begin to develop, pigment cell progenitors contribute new melanophores as well (5.9 SSL) (Parichy et al., 2003b, 2009). Newly melanizing cells are evident in prospective stripe and interstripe regions. As pattern implementation continues, additional, morphologically distinct iridophores appear within the prospective stripes.

Subsequently, the initial pattern of an interstripe and two stripes becomes more distinctive. This depends on a consolidation of the melanophores into stripe regions: melanophores differentiating outside of these regions—and a few EL melanophores at the horizontal myoseptum—either migrate short distances to join stripes, or they die or are obscured by iridophores (Parichy et al., 2000, 2003b; Patterson et al., 2013; Takahashi et al., 2008). Several studies have revealed the importance of iridophores in promoting melanophore localization to stripe regions, and excluding these cells from the interstripe itself (Fadeev et al., 2015; Frohnhofer et al., 2013; Krauss et al., 2013; Patterson et al., 2013, 2014).

Two events occur nearly simultaneously with stripe consolidation and are important to this process. First, new iridophores appear and become increasingly dense

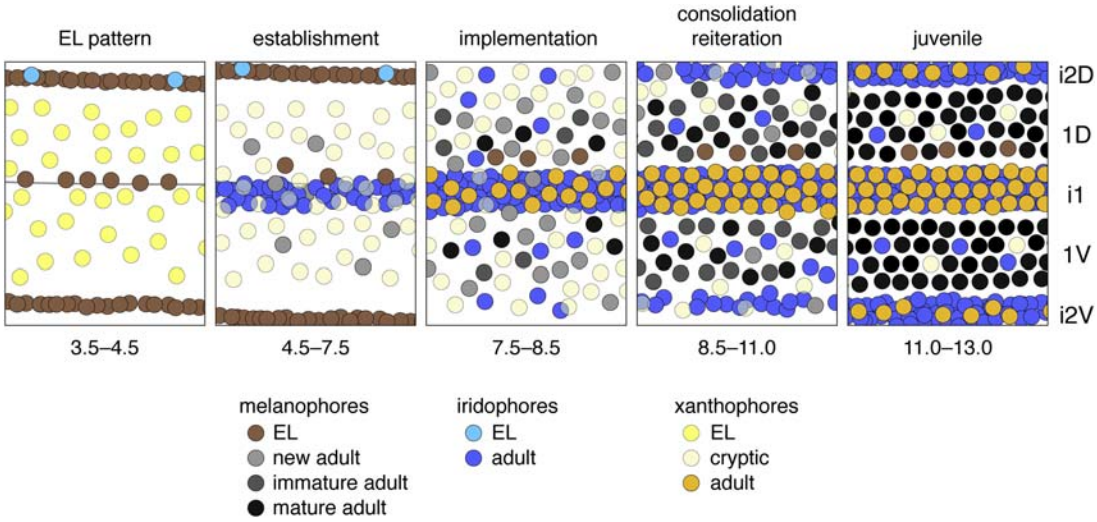

FIGURE 9.2 Events of adult pattern formation. In the EL pattern, melanophores, xanthophores, and a few iridophores are present. *Gray line* indicates horizontal myoseptum. By the establishment of the adult pattern, the fish is growing rapidly, and EL xanthophores have faded, entering a cryptic state. Some EL melanophores move, and some are lost. New adult iridophores start to differentiate, and new adult melanophores begin to appear. During a period of pattern implementation, adult melanophores increase in number, xanthophores acquire new pigment, and iridophores appear within the prospective stripe regions. Consolidation of stripes occurs with the onset of pattern reiteration. Ultimately, a juvenile pattern is formed, which persists with some additional reiteration in the adult. i1: primary interstripe; 1D, 1V: primary stripes; i2D, i2V, secondary interstripes. Ranges below panels are SSL (Parichy et al., 2009).

within new, "secondary" interstripes that develop adjacent to the primary stripes and serve to bound their "distal" edges (Patterson et al., 2013, 2014; Singh et al., 2014; Spiewak et al., 2018). Second, xanthophores differentiate in association with iridophores of the primary interstripe (~8.0 SSL) owing to a xanthophore-maturation factor produced by these iridophores (Patterson et al., 2013). Iridophores, therefore, contribute to the arrangement of pigmented xanthophores, in addition to their effects on melanophores. Xanthophores, in turn, influence the localization and survival of melanophores and are essential for normal consolidation and subsequent maintenance of the stripes (Hamada et al., 2014; Nakamasu et al., 2009; Parichy et al., 2000, 2003a). Both xanthophores and melanophores additionally depend on permissive factors provided by other integumentary cell types (Hultman et al., 2007; Patterson et al., 2013).

The origin of many xanthophores differs from adult melanophores and adult iridophores. Rather than arising from a postembryonic pigment cell progenitor, many pigmented xanthophores of the adult come directly from xanthophores of the EL pattern. As noted above, these cells fade from view, but they also proliferate and persist during subsequent stages, and it is some of these cells that reacquire pigmentation, when in association with interstripe iridophores (McMenamin et al., 2014). The color of xanthophores at EL stages is due to yellow pteridines (Lister, 2019; Odenthal et al., 1996). Their color in the adult results from the thyroid-hormone dependent processing and accumulation of dietarily derived yellow/orange carotenoids (Granneman et al., 2017; Saunders et al., 2019). Besides xanthophores that develop directly from the neural crest and EL xanthophores, at least some adult xanthophores develop from postembryonic pigment cell progenitors (McMenamin et al., 2014; Singh et al., 2016).

Together, these events are responsible for a primary pattern consisting of an interstripe bordered by two stripes. As the fish grow, this pattern is reiterated with increasingly well-defined secondary interstripes and stripes, added dorsally and ventrally. Just as interactions between pigment cells are required for patterning the primary pattern elements, interactions between pigment cells, and between pigment cells and other cells in their environment, are required for patterning the secondary elements (Parichy et al., 2003a; Patterson et al., 2013, 2014; Spiewak et al., 2018). The overall dynamics of stripe development resemble those predicted by Turing models of pattern formation (Watanabe et al., 2015); grounding such similarities in discrete biological mechanisms remains a substantial and important challenge.

By late juvenile stages (~16 SSL), a flank pattern of stripes and interstripes has formed that will persist into the adult (≥26 SSL). Also by juvenile and adult stages, pigment cells comprising this pattern have become stratified: xanthophores are outermost, and iridophores, then melanophores, are found inwardly. An additional, less studied, population of spindle-shaped iridophores with large reflecting platelets occurs in smaller numbers even deeper in the hypodermis (Hirata et al., 2003, 2005).

Scales, Fins, and Other Sites of Pigmentation

As zebrafish enter the juvenile stages of development, they have transparent scales covering their bodies ((Parichy et al., 2009); Aman & Parichy, this volume). The same multipotent pigment cell progenitors that give rise to many chromatophores of the hypodermal stripes and interstripes are responsible for populating scales with chromatophores; indeed, individual progenitors can contribute to both locations (Singh et al., 2016). Melanophores differentiate prominently on the dorsal scales, but these cells are repressed from differentiating ventrally by the agouti signaling pathway, which has a similar function in repressing the differentiation of melanocytes on the ventrum of many mammals (Cal et al., 2019).

Pigmentation of adult fins begins as soon as they develop; pattern outcomes differ between anatomical locations (Parichy et al., 2009). In the caudal and anal fins, stripes and interstripes develop, but their arrangements are independent of iridophores, which occur in relatively small numbers at these sites. The dorsal and paired fins have similar complements of chromatophores, but the cells remain largely intermingled and so do not generate distinct patterns.

Fins also harbor leucophores (Lewis et al., 2019). Distal regions of the dorsal fin and the most distal portions of the caudal fin lobes develop leucophores that arise by transdifferentiation of melanophore progenitors. These cells lose melanin, and in its place, acquire crystalline deposits of guanine. These "melanoleucophores" are white owing to a disordered arrangement of irregularly shaped organelles containing guanine crystals. This contrasts with the iridescence conferred by stacked, regularly shaped reflecting platelets of guanine crystals in iridophores. Melanoleucophores reflect light of all wavelengths: they are bright in visible light, and the reflections from these cells can be mistaken for the fluorescence of transgenic reporters. Due to their prominent locations, these cells may contribute to species recognition, or aggressive or courtship displays. In the laboratory, zebrafish prefer to shoal with fish that have intact complements of melanoleucophores. The second class of leucophores, "xantholeucophores," is found in the interstripes of the anal fin, and contains yellow/orange carotenoids, similar to xanthophores. Crystalline guanine is not detectable in xantholeucophores.

Finally, zebrafish also have chromatophores in other locations, including iridophores that line the peritoneum and cover the operculum, and cells of the choroid and iris of the eye (retinal pigmented epithelium derives from the central nervous system) (Hirata et al., 2005; Spiewak et al., 2018). Little is known about the development or functional significance of these features.

Physiological and Pathological Effects on Pigmentation

Pigmentation changes ontogenetically, but pigment cells also respond physiologically to alterations of environment or health status. A normal physiological response occurs in background adaptation. When fish are placed on a light background, melanophores contract melanin-containing melanosomes toward their centers, resulting in an overall paler appearance to the fish. On a dark background, the opposite response occurs. Such behaviors depend on endocrine and neuroendocrine effectors and have been studied extensively in zebrafish and other species (Counts et al., 2009; Fujii, 1993; Iwashita et al., 2006; Lewis et al., 2019; Oshima et al., 2002; Sheets et al., 2007). Although responses are physiological, long-term stimulation can lead to morphological alterations resulting from cell death or overproduction (Sugimoto, 2002; Sugimoto et al., 2005).

Pigmentation can also change in response to stress or pathology, sometimes mimicking the blanching response of healthy fish adapted to a light background. Other pathologies can yield dark phenotypes. Additionally, injuries sometimes result in pigmentary "scars." Zebrafish have a remarkable ability to heal integumentary wounds, but deep wounds, trauma, or inflammatory responses can generate ectopic accumulations of chromatophores (Levesque et al., 2013; Richardson et al., 2013) Bilateral asymmetry typically distinguishes such marks from pattern phenotypes arising through genetic alterations.

Conclusions

Studies of zebrafish pigmentation have provided insights into mechanisms of pattern formation, specification, and differentiation of neural crest lineages, cellular physiology, and individual behavior. Pigmentation can also provide clues to fish health and physiology. Due to the superficial location of chromatophores, their accessibility to visualization, and their cell-autonomous markers of differentiation state, this system should continue to be useful for understanding the basic and applied aspects of organismal form and function.

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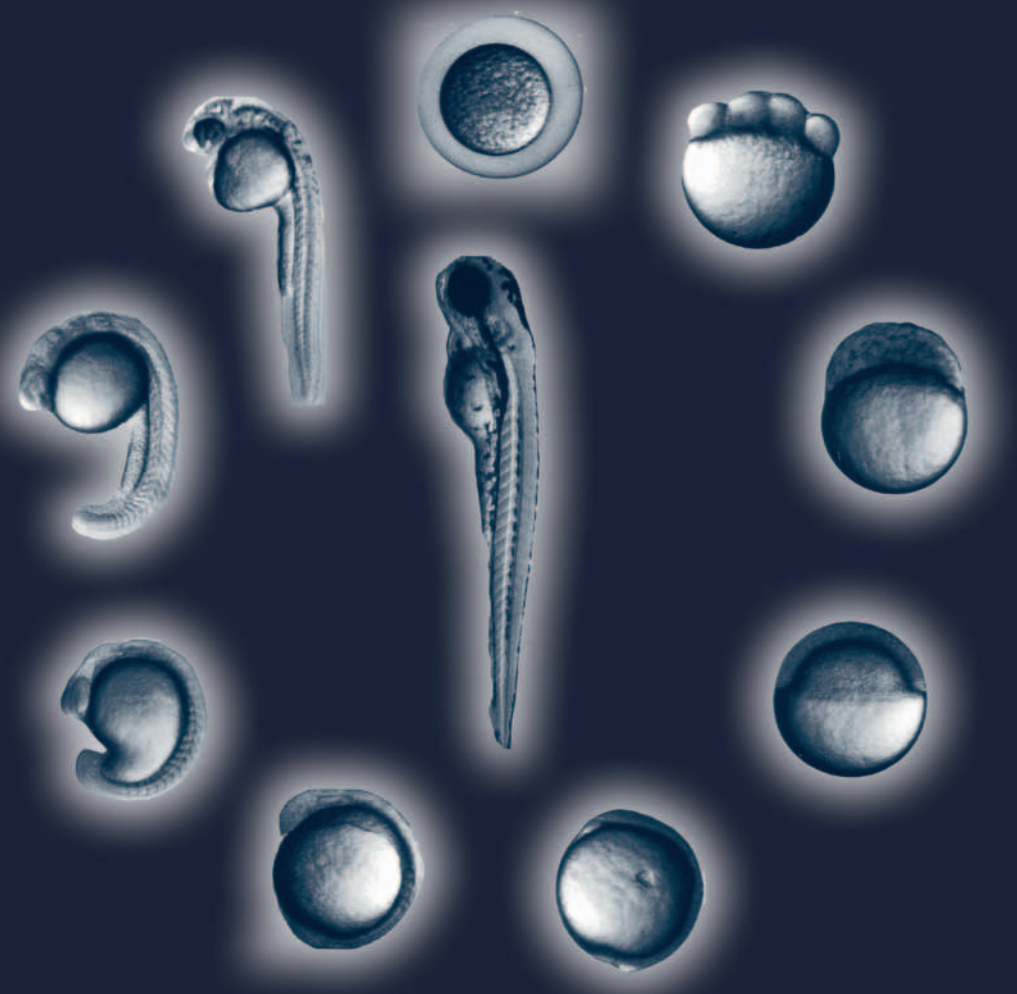

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American College of Laboratory Animal Medicine Series

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