Developmental Biology **240,** 301–314 (2001)
doi:10.1006/dbio.2001.0418, available online at http://www.idealibrary.com on IDEAL®

REVIEW

How the Zebrafish Gets Its Stripes

John F. Rawls, Eve M. Mellgren, and Stephen L. Johnson[1]

Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110

The study of vertebrate pigment patterns is a classic and enduring field of developmental biology. Knowledge of pigment pattern development comes from a variety of systems, including avians, mouse, and more recently, the zebrafish (*Danio rerio*). Recent analyses of the mechanisms underlying the development of the neural crest-derived pigment cell type common to all vertebrates, the melanocyte, have revealed remarkable similarities and several surprising differences between amniotes and zebrafish. Here, we summarize recent advances in the study of melanocyte development in zebrafish, with reference to human, mouse, and avian systems. We first review melanocyte development in zebrafish and mammals, followed by a summary of the molecules known to be required for their development. We then discuss several relatively unaddressed issues in vertebrate pigment pattern development that are being investigated in zebrafish. These include determining the relationships between genetically distinct classes of melanocytes, characterizing and dissecting melanocyte stem cell development, and understanding how pigment cells organize into a patterned tissue. Further analysis of zebrafish pigment pattern mutants as well as new generations of directed mutant screens promise to extend our understanding of pigment pattern morphogenesis. © 2001 Elsevier Science

Key Words: **zebrafish; *Danio rerio*; neural crest; melanocyte; melanophore; stem cell; pigment pattern.**

INTRODUCTION

Vertebrate pigment patterns have captivated biologists and laypersons for centuries. Over the years, this has resulted in large collections of pigment pattern variants or mutations in a variety of species, such as those of the mouse fanciers in the 19th century which helped lay the foundations of mouse genetics (Silver, 1995). The inherent labeling of pigment cells also facilitated many of the earliest advances in vertebrate developmental genetics, including the first demonstrations of Mendelian genetics in animals (Cuénot, 1908; Castle and Little, 1910; Wright, 1917), and helped to reveal the remarkable migratory properties of neural crest cells (see Horstadius, 1950). Similar to the early mouse collections, many of the earliest described mutations in the zebrafish *Danio rerio*, including Streisinger and Walker's first pigment pattern mutations (*albino*^{b1}, *golden*^{b2}, *brass*^{b4}, *sparse*^{b5}; Streisinger *et al.*, 1986), *leopard*^{t1} (Kirschbaum, 1975; Johnson *et al.*, 1995b), and *panther*^{j4blue} (Parichy *et al.*, 2000b), were recovered from pet store stocks. Among these pigment pattern mutations, those that affect the pigment cell type common to both amniotes and fishes, the melanocyte, have attracted the most attention over the years.

The study of melanocyte development in model organisms is critical for the understanding and treatment of human melanocyte disorders such as vitiligo, piebaldism, Waardenburg Syndrome, and melanoma (reviewed in Nordlund *et al.*, 1998). Knowledge of the mechanisms underlying melanocyte development may also improve our understanding of development of other neural crest derivatives, and provide insight into general mechanisms of cell migration, survival, differentiation, and fate specification. A good conceptual framework for understanding melanocyte development has been provided by studies in avians (primarily using embryonic manipulation and lineage analysis) and mice (primarily through identifying genes and testing gene function). The zebrafish *Danio rerio* provides another opportunity to dissect the mechanisms underlying many different aspects of vertebrate development. The accessibility and transparency of the developing embryo combined with the capacity for forward mutational analysis has allowed zebrafish to quickly become a preferred vertebrate

[1] To whom correspondence should be addressed at: Campus Box 8232, 4566 Scott Avenue, St. Louis, MO 63110. Fax: (314) 362-7855. E-mail: sjohnson@sequencer.wustl.edu.

0012-1606/01 $35.00
© 2001 Elsevier Science
All rights reserved.

FIG. 1. Zebrafish mutants affect distinct classes of adult stripe melanocytes. Adult wild-type zebrafish (WT) have melanocyte stripes in the body, and in the caudal and anal fins. Adult homozygous mitf^{w2} (*nacre*) mutants [*mitf* (*nacre*)] lack all neural crest-derived melanocytes. Several zebrafish mutants develop around half the normal complement of adult body stripe melanocytes, lacking either early stripe melanocytes (ESMs) or late stripe melanocytes (LSMs). Homozygous *fms*^{j4e1} (*panther*) mutants [*fms* (*panther*)] and *ednrb1*^{b140} (*rose*) mutants [*ednrb1* (*rose*)] lack LSMs, while *kit*^{b5} (*sparse*) mutants lack ESMs and dorsal scale-associated melanocytes [*kit* (*sparse*)]. Adults homozygous for both *kit*^{b5} and *ednrb1*^{b140} lack all body stripe melanocytes (*kit;ednrb1*), demonstrating that the melanocyte classes absent in each of the respective single mutants are genetically distinct. Note that the *kit;ednrb1* double mutants retain melanocyte stripes in the caudal and anal fins, revealing a third class of adult melanocytes.

developmental system. These benefits also apply to analysis of the zebrafish embryonic pigment pattern and the characteristic striped adult pigment pattern.

Zebrafish Melanocyte Development

During vertebrate embryogenesis, neural crest cells arise along the dorsal neural tube, disperse throughout the body, and differentiate into several distinct cell types, including sensory and sympathetic neurons, Schwann cells, and pigment cells (Weston, 1970; LeDouarin, 1982; Raible *et al.*, 1992; Groves and Bronner-Fraser, 1999). Several different types of pigment cells arise from the zebrafish neural crest, including yellow xanthophores, reflective iridophores, and black melanocytes (sometimes referred to as melanophores in poikilotherms; reviewed in Bagnara, 1998). Specification of neural crest cell fate in zebrafish usually occurs prior to migration (Raible and Eisen, 1994), suggesting that the signals promoting the melanocyte fate initiate very early in development. Presumptive melanocyte precursors (melanoblasts) begin expressing melanin pigment around 24 h postfertilization, often before completing migration (Raible *et al.*, 1992). The embryonic melanocyte pattern is largely completed by 48 h postfertilization, and only minimal addition and loss of melanocytes occur over the next 12 days of larval development (Milos and Dingle, 1978a,b; J.F.R. and S.L.J., unpublished data).

The larval pigment pattern established during the first few days of development is gradually replaced by the adult pigment pattern during the larval-to-adult metamorphosis (2–4 weeks; Johnson *et al.*, 1995b). Beginning at 2 weeks, stripe melanocytes begin to appear evenly distributed in the flank, then subsequently coalesce into the first body stripes over the next 2 weeks. This first wave of adult melanocytes (early stripe melanocytes, ESMs) is followed at 3 weeks by a marked increase in the number of new stripe melanocytes (late stripe melanocytes, LSMs), and the appearance of scale-associated melanocytes on the dorsum of the animal (Johnson *et al.*, 1995b). Similar stripes form in the caudal and anal fins during this time, but by somewhat different genetic mechanism (see below; Rawls and Johnson, 2000). Following establishment of the adult pattern (Fig. 1) during the first month of life, existing stripes are maintained and new stripes are gradually added dorsally and ventrally as the animal grows.

While mechanisms underlying growth and homeostasis

© 2001 Elsevier Science. All rights reserved.

TABLE 1
Conserved and Divergent Gene Requirements in Vertebrate Melanocyte Development

Gene	Conserved developmental requirements	Divergent developmental requirements
Wnt1/Wnt3a	Neural crest and melanocyte specification	N/A
Sox10	Melanocytes, peripheral neurons, and glia	Fish: xanthophores, iridophores
Mitf	Embryonic and adult melanocytes	Mice: RPE,[a] fish: embryonic xanthophores and iridophores
Kit/Slf	Embryonic and adult melanocytes	Mice: hematopoietic precursors,[a] PGCs
Ednrb/Et3	Adult melanocytes	Mice: embryonic melanocytes, fish: adult iridophores
Fms/Csf1	Osteoclasts	Mice: macrophages;[a] fish: adult melanocytes, embryonic and adult xanthophores

[a] Although gene requirements have not been conserved in these cell types, gene expression has been conserved.

of established body stripes is unclear, it is tempting to speculate that stem cells provide new melanocytes to the stripe as it broadens or to replace dead cells. Evidence for such mechanisms comes from studies of regenerating fin stripes. Following partial amputation of the caudal or anal fin, adult fish fins regenerate to entirely replace the missing structure including the characteristic melanocyte stripes (Goodrich and Nichols, 1931; Johnson and Weston, 1995; Johnson and Bennett, 1999). Indirect lineage analysis suggests that the melanocytes that reestablish the fin stripes during regeneration arise from self-renewing unpigmented precursors, or stem cells (Rawls and Johnson, 2000). Mutational analysis similarly suggests that unpigmented precursors produce the melanocytes of the adult body stripes during the larval-to-adult pattern metamorphosis (Johnson *et al.*, 1995b). Taken together, these results raise the possibility that melanocyte stem cells are responsible for morphogenesis and homeostasis of all components of the adult zebrafish melanocyte pattern. Furthermore, the recent description of melanocyte stem cells in adult mice that produce new melanocytes during the hair cycle (Slominski *et al.*, 1996; Kunisada *et al.*, 1998; Botchkareva *et al.*, 2001) suggests that development and homeostasis via stem cells might be an evolutionarily conserved strategy in pigment pattern development.

Mammalian Melanocyte Development

Development of the melanocyte lineage in mammals (reviewed in Quevedo and Holstein, 1998) is similar to zebrafish, with some notable exceptions. The mammalian pigment pattern is generated exclusively by melanocytes, unlike that of zebrafish which contains several types of pigment cells. Following dispersal from the neural crest, mammalian embryonic melanoblasts typically cross the basement membrane into the epidermis. Some then remain dispersed in the epidermis, while others localize to the hair follicles. In contrast, fish melanocytes typically remain beneath the basement membrane, unless extruded through the skin during apoptosis (Parichy *et al.*, 1999; Sugimoto *et al.*, 2000). Also in contrast to zebrafish, mammalian melanoblasts do not express melanin until they complete migration, although expression of earlier melanoblast markers initiates prior to crossing the basement membrane (Orr-Urtreger *et al.*, 1990; Steel *et al.*, 1992). Once melanin is formed in mammalian melanocytes, it is packaged into melanosomes and transferred to other cells, such as keratinocytes of developing hair or neighboring epidermal cells. Instead of exporting their melanosomes to other cells, fish melanocytes retain their melanosomes which can be redistributed throughout the cell via transport along a microtubule network (McNiven and Porter, 1984; Jimbow and Sugiyama, 1998).

CONSERVED AND DIVERGENT ROLES OF GENES IN PIGMENT CELL DEVELOPMENT

Progress in identifying genes underlying mouse coat color mutants has facilitated the identification of their orthologs in zebrafish. These genes have provided many successful candidates for zebrafish pigment pattern mutants, or allowed more directed analysis of their roles in zebrafish pigment pattern development. Comparison of the roles of these genes in fish and amniotes reveals many similarities and several surprises. These are briefly summarized in Table 1, and discussed below. An emerging challenge is to determine whether differences between the developmental requirements for a gene in fish and amniotes is caused by divergence of gene function between taxa, or by functional redundancy between the gene and a duplicated paralog produced through partial or complete genome duplication (Postlethwait *et al.*, 1998; Barbazuk *et al.*, 2000).

We follow standard nomenclature for genes and proteins (i.e., human: *KIT* and KIT; mouse: *Kit* and Kit; zebrafish: *kit* and Kit), except when discussing multiple species we default to mouse terminology (i.e., *Kit* and Kit). For zebrafish mutants that have not been renamed for their encoded gene (i.e., *nacre* = *mitf*), we use the name of the gene whenever possible.

The Wnt Pathway

Among the earliest steps in formation of the neural crest and specification of the melanocyte lineage is signaling

© 2001 Elsevier Science. All rights reserved.

through the Wnt family of cysteine-rich secreted glycoproteins and their downstream effectors, including β-catenin and Tcf/Lef transcription factors (for review see Christiansen *et al.*, 2000; Dorsky *et al.*, 2000a). While mice mutant for either *Wnt1* or *Wnt3a* develop neural crest cells and their derivatives normally (McMahon and Bradley, 1990; Thomas and Capecchi, 1990; Takada *et al.*, 1994), mice doubly mutant for both *Wnt1* and *Wnt3a* develop fewer neural crest cells including deficiencies in melanoblasts (Ikeya *et al.*, 1997). Although zebrafish mutations in these genes are not yet identified, ectopic expression of Wnt-1 in zebrafish (Dorsky *et al.*, 1998) and Wnt-1 or Wnt-3a in *Xenopus* (Saint-Jeannet *et al.*, 1997) causes an increase in the number of cells expressing neural crest markers. This increase can occur when cell division is inhibited (Saint-Jeannet *et al.*, 1997), suggesting that this Wnt signaling event may play a role in specification of neural crest fate rather than in neural crest precursor proliferation.

In addition to early roles in neural crest specification, Wnt signaling also promotes specification of neural crest-derived pigment cells. Activation of Wnt signaling in single neural crest cells in zebrafish (Dorsky *et al.*, 1998) or treatment of avian neural crest cultures with Wnt-conditioned medium (Jin *et al.*, 2001) has been shown to promote pigment cell fates at the expense of neural and glial fates. Wnt signaling may also act to expand the melanocyte lineage, or promote differentiation, as revealed by targeted activation of Wnt signaling in murine melanoblasts cells (Dunn *et al.*, 2000). Taken together, these results place Wnt signaling at several early stages in melanocyte fate specification and development. Determining how Wnt signaling promotes melanocyte development may be accomplished through identification of downstream effectors or transcription factors, such as Mitf (Dorsky *et al.*, 2000b; Takeda *et al.*, 2000)

Sox10

The role of the *Sox10* gene, that encodes a SRY-related HMG transcription factor, in melanocyte development was revealed by its identification as the affected gene in Waardenburg syndrome type 4 patients (Pingault *et al.*, 1998) and in the *Dominant megacolon* mutant in mice (Southard-Smith *et al.*, 1998). Loss of *Sox10* function in mammals is characterized by deficiencies in neural crest-derived melanocytes and components of the peripheral nervous system, including enteric neurons and glia (Pingault *et al.*, 1998; Southard-Smith *et al.*, 1998; Inoue *et al.*, 1999; Pingault *et al.*, 2000; Britsch *et al.*, 2001). Recent work has shown that a zebrafish ortholog of *Sox10* corresponds to the zebrafish *colourless* mutant (Dutton, *et al.*, 2001). Zebrafish *sox10* mutants show severe reductions in a subset of neural crest derivatives, including enteric neurons and glia, and all three types of pigment cells (Kelsh and Eisen, 2000; Kelsh *et al.*, 2000). These studies show that *Sox10* is required for the development of a subset of neural crest fates, including melanocytes, and that developmental requirements for *Sox10* have been largely conserved between fish and mammals.

Mitf

The basic helix–loop–helix/leucine zipper protein Mitf (microphthalmia-associated transcription factor) has been implicated as a key regulator of the melanocyte lineage (for review see Moore, 1995; Goding, 2000; Tachibana, 2000). Mammalian Mitf directly binds promoter elements and promotes the transcription of a variety of genes, including the *Kit* receptor tyrosine kinase (Tsujimura *et al.*, 1996; Opdecamp *et al.*, 1997) and members of the tyrosinase family of melanin synthesis enzymes (Bentley *et al.*, 1994; Hemesath *et al.*, 1994; Yasumoto *et al.*, 1994, 1995, 1997). Mice carrying strong hypomorphic *Mitf* alleles have severe deficits in neural crest-derived melanocytes, as well as microphthalmia, osteopetrosis, and deafness (Hodgkinson *et al.*, 1993). In humans, mutations in *MITF* have been linked to Waardenburg syndrome 2, a disorder characterized by melanocyte deficits and deafness that ensues from inner ear melanocyte deficiencies (Tassabehji *et al.*, 1994). Zebrafish *nacre* mutants, which lack all neural crest-derived melanocytes (Fig. 1) and have excess iridophores, result from lesions in a zebrafish ortholog of *Mitf* (Lister *et al.*, 1999). Zebrafish *mitf* is expressed by precursors of both the melanocyte and xanthophore lineage, raising the possibility that *mitf* is involved in the development of both pigment cell types (Parichy *et al.*, 2000b). The ability of *Mitf* overexpression to cause cells to adopt melanocyte characteristics in both fish (Lister *et al.*, 1999) and mice (Tachibana *et al.*, 1996) raises the possibility that *Mitf* expression could be sufficient as well as necessary for melanocyte development. While these results taken together suggest roles for *Mitf* in promoting early stages of melanocyte development, *Mitf* may also have later roles in melanocyte proliferation or survival (Lerner *et al.*, 1986). The finding that zebrafish *mitf* mutants retain retinal pigmented epithelia (RPE) while mice mutant for *Mitf* lack RPE and have other eye defects (Hodgkinson *et al.*, 1993; Moore, 1995), raises the possibility that a second zebrafish *mitf* ortholog might function redundantly with *nacre* to promote development of the RPE (Lister *et al.*, 2001).

Multiple melanocyte specification pathways converge in the control of *Mitf* transcription (Bondurand *et al.*, 2000; Fisher, 2000; Tachibana, 2000). First, *Mitf* expression in humans and zebrafish is transcriptionally regulated by the Wnt pathway through Tcf/Lef binding sites in the *Mitf* promoter (Dorsky *et al.*, 2000b; Takeda *et al.*, 2000). Second, Sox10 has been shown to bind *Mitf* regulatory sequences in human and mouse, and consequently activate *Mitf* transcription (Lee *et al.*, 2000; Potterf *et al.*, 2000; Verastegui *et al.*, 2000). Also, melanocyte-stimulating hormone in mouse has been shown to promote *Mitf* transcription presumably through increased cAMP levels and a cAMP response element in the *Mitf* promoter (Bertolotto *et*

© 2001 Elsevier Science. All rights reserved.

al., 1998; Busca and Balotti, 2000). Another potential regulator of *Mitf* expression is the paired homeodomain transcription factor Pax3, which is required for development of the neural tube, melanocytes and other neural crest derivatives in mammals (Epstein *et al.,* 1991; Tassabehji *et al.,* 1992; Hoth *et al.,* 1993; Serbedzija and McMahon, 1997). Pax3 can bind to the *Mitf* promoter and activate its transcription in both mice (Potterf *et al.,* 2000) and humans (Watanabe *et al.,* 1998), although it remains possible that the requirement for Pax3 in melanocyte development is indirect (Hornyak *et al.,* 2001). Further analysis will have to address possible relationships between these transcriptional regulators of *Mitf*, and the multiple *Mitf* isoforms (Hallsson *et al.,* 2000; Shibahara *et al.,* 2000) and promoters (Yasumoto *et al.,* 1998; Udono *et al.,* 2000) found in mammals.

Kit and Slf

The Kit type III receptor tyrosine kinase (Kit) and its ligand, Steel factor (Slf), are also required for vertebrate melanocyte development (for review see Besmer *et al.,* 1993). In the absence of Kit or Slf function, embryonic melanoblasts in mammals are unable to migrate and subsequently die (Motro *et al.,* 1991; Morrison-Graham and Weston 1993; Cable *et al.,* 1995; Wehrle-Haller and Weston, 1995; Bernex *et al.,* 1996; MacKenzie *et al.,* 1997; Ito *et al.,* 1999; Wehrle-Haller *et al.,* 2001). Zebrafish mutant for a *Kit* ortholog (*sparse*) have a similar embryonic melanocyte defect. Although melanocytes differentiate in the zebrafish *kit* mutant embryo, they largely fail to migrate and subsequently undergo programmed cell death (Parichy *et al.,* 1999). One interesting difference in the roles of *Kit* in mammals and fish is in primordial germ cell (PGC) and blood development. Mice mutant for either *Kit* or *Slf* lack PGCs and die from macrocytic anemia (Mintz and Russell, 1957; Russell, 1979; Broudy, 1997; Sette *et al.,* 2000). Zebrafish *kit* is not expressed in PGCs, is expressed in the blood lineage, and null mutants are fully fertile and nonanemic (Parichy *et al.,* 1999).

While the embryonic phenotypes of *Kit* mutants reveal roles for *Kit* in early melanoblast dispersal and survival, loss of *Kit* function in fish and mammals also causes deficits in adult melanocytes (Silvers, 1979; Geissler *et al.,* 1988; Nocka *et al.,* 1990; Tan *et al.,* 1990; Giebel and Spritz, 1991; Tsujimura *et al.,* 1991; Marklund *et al.,* 1998; Botchkareva *et al.,* 2001). In mice, injections of Kit-directed antibodies result in coat pattern phenotypes, which suggest that Kit and Slf promote proliferation of melanoblasts in the mesoderm and their colonization of the epidermis (Nishikawa *et al.,* 1991; Yoshida *et al.,* 1996). Once melanoblasts are in the epidermis, Slf may serve as a motogenic signal to promote melanoblast colonization of the hair follicle (Jordan and Jackson, 2000). Zebrafish mutant for *kit* also display pigment pattern defects during later developmental stages. Following loss of all embryonic melanocytes, juvenile *kit* null mutants fail to form ESMs at 2 weeks, but then develop LSMs at 3 weeks to produce an adult stripe pattern that consists of about half of the wild-type complement of melanocytes (Fig. 1; Johnson *et al.,* 1995b). Additionally, zebrafish *kit* mutants fail to develop the melanocytes that normally arise in the regenerating fin beginning at 4 days after amputation (primary fin regeneration melanocytes). Instead, *kit* mutant fin stripes are reestablished beginning around 8 days after amputation by a secondary population of regeneration melanocytes (secondary fin regeneration melanocytes; Rawls and Johnson, 2000). As both adult body stripe and fin stripe melanocytes arise from stem cells, these melanocyte deficits in *kit* mutants suggest that *kit* is important in melanocyte stem cell development (see below).

In addition to the numerous developmental requirements for *Kit* described above, a growing set of signaling molecules that act downstream from *Kit* have been identified (for review see Linnekin, 1999; Taylor and Metcalfe, 2000). An emerging challenge is to determine the relationships between different downstream signaling pathways and distinct developmental roles for *Kit*. For example, it remains unclear whether embryonic melanocytes in *Kit* mutants are unable to migrate because they are dying, whether they die because they are unable to migrate, or whether *Kit* promotes migration and survival independently. Furthermore, the downstream signaling molecules responsible for mediating *Kit*-dependent survival and migration have yet to be fully elucidated. Toward this goal, *in vivo* studies indicate that *Kit*'s role in embryonic melanoblast migration in mice is separable from its role in survival, and that its role in survival is mediated by the RAS pathway (Wehrle-Haller *et al.,* 2001). The recent identification of zebrafish *kit* alleles that specifically disrupt either melanocyte migration or survival should provide further insight into relationships between the structure of the Kit receptor and its separable developmental roles (J.F.R. and S.L.J., unpublished data).

A complete understanding of the genes required for melanocyte development should also include the temporal order in which these genes function. The complexity of this challenge has been illustrated by efforts to understand the relationship between *Kit* and *Mitf* function. One model for this temporal relationship is that melanocyte precursors first express *Mitf*, which leads to expression of *Kit* in a subset of pigment cells (Parichy *et al.,* 2000b). This is supported by the observations that *Mitf* is required to upregulate *Kit* expression (Opdecamp *et al.,* 1997), and that Mitf can bind the *Kit* promoter and activate its transcription (Tsujimura *et al.,* 1996). This model is also supported by observations that zebrafish *mitf* mutants fail to develop embryonic melanocytes (Lister *et al.,* 1999) while *kit* mutants form embryonic melanocytes that fail to migrate and eventually undergo apoptosis (Parichy *et al.,* 1999). Another possible model is that initial expression of *Kit* and signaling through the Kit receptor leads to the transcriptional activation of *Mitf*. This model is supported by mouse cell culture experiments showing that a downstream component of the Kit signaling pathway, Erk2, phosphorylates Mitf, thereby

© 2001 Elsevier Science. All rights reserved.

creating a binding site for the transcriptional coactivator p300 (Sato *et al.*, 1997; Hemesath *et al.*, 1998; Price *et al.*, 1998). This transcriptional activation may be transient, however, because signaling through Kit can simultaneously target Mitf for both transcriptional activation and ubiquitin-mediated degradation (Wu *et al.*, 2000). A third model posits that *Mitf* and *Kit* function cooperatively to perform some tasks in the melanocyte lineage and function independently to fulfill other roles. Consistent with this possibility, Hou and coworkers (2000) have shown that *Mitf* expression without Kit function is sufficient for expression of the melanin synthesis enzyme genes *Dct* and *Trp1*, but that Kit signaling is required for the expression of the *Tyr* gene, encoding the rate-limiting enzyme in melanin synthesis.

Ednrb and Et3

Another signaling pathway important in melanocyte development is that mediated by the G-protein coupled endothelin receptor B (Ednrb) and its ligand endothelin 3 (Et3). Mutational analysis in mice has shown that Ednrb signaling is essential for neural crest-derived melanocytes and enteric neurons (Baynash *et al.*, 1994; Hosoda *et al.*, 1994; Yanagisawa *et al.*, 1998). Similarly, mutations in human EDNRB or ET3 have been shown to cause pigmentary defects and aganglionic megacolon found in Waardenburg syndrome and Hirschsprung disease (Puffenberger *et al.*, 1994; Attie *et al.*, 1995; Chakravarti, 1996; Edery *et al.*, 1996; Hofstra *et al.*, 1996). Studies suggest that the Ednrb pathway is required for proliferation, differentiation, and survival of early neural crest-derived melanocyte precursors (Lahav *et al.*, 1998; Shin *et al.*, 1999) and differentiation of epidermal melanocytes (Reid *et al.*, 1996; Dupin *et al.*, 2000). The zebrafish pigment pattern mutant *rose* corresponds to a zebrafish ortholog of *Ednrb* (*ednrb1*). Zebrafish *ednrb1* is expressed by melanocytes, iridophores, and xanthophores in the embryo, and expressed by melanocytes and iridophores during the larval-to-adult metamorphosis (Parichy *et al.*, 2000a). Unlike mammals, zebrafish *ednrb1* mutants do not display defects in pigment pattern development during embryonic and larval stages, and form ESMs normally. However, *ednrb1* mutants fail to form LSMs, resulting in an adult body stripe pattern that consists of only ESMs (~50% of the wild-type complement of melanocytes; Fig. 1; Johnson *et al.*, 1995b). These differences between *Ednrb* requirements in amniotes and zebrafish could reflect either redundant roles for the zebrafish ortholog during embryogenesis, or divergence of *Ednrb* function between these taxa.

Fms

The roles of genes in melanocyte development discussed above are largely comparable between zebrafish and mammals. An exception is the *Fms* type III receptor tyrosine kinase (also called *Csf1r*), which is required for pigment pattern development in zebrafish but for which no role has been described in mouse. Zebrafish *fms* (*panther*) mutants lack embryonic and adult xanthophores, as well as LSMs of the adult body stripes (Fig. 1; Parichy *et al.*, 2000b). Zebrafish *fms* is also expressed in macrophage progenitors and both expressed by and required for development of osteoclasts. Although *Fms* mutant mice have not been described, mutants for its ligand, *Csf1* (also called M-CSF), have deficits in macrophages and osteoclasts (Felix *et al.*, 1994; Flanagan and Lader, 1998; Motoyoshi, 1998), but have no defects in pigment pattern or other neural crest derivatives (Marks and Lane, 1976). Therefore, *Fms* has phylogenetically conserved expression in macrophages and osteoclasts, but has divergent roles in pigment pattern morphogenesis. Since *fms* has been implicated in pigment pattern diversification between zebrafish (*Danio rerio*) and pearl danio (*Danio albolineatus*), its role in pigment pattern may not be unique to zebrafish (Parichy and Johnson, 2001).

OPPORTUNITIES IN PIGMENT PATTERN DEVELOPMENTAL BIOLOGY

Diversification of Tissue Types

One striking difference between pigment pattern development in fish and mice is the presence of multiple melanocyte classes in fish (see Table 2). While all melanocytes in mouse belong to a single class that requires *Kit* and *Endrb* function, genetic and developmental analysis in zebrafish has revealed several classes of melanocytes in the adult body pattern with distinct gene requirements. Mutants in *kit*, *ednrb1*, *fms*, *leopard*, or *primrose* each develop approximately half of the normal complement of adult body stripe melanocytes (Fig. 1; Johnson *et al.*, 1995b; Parichy *et al.*, 2000b; S.L.J., unpublished data). Epistasis analyses show that these pigment pattern mutations can be placed into two epistasis groups. One group consists of only one gene, *kit*, which is required for the development of ESMs but not LSMs. In contrast, the other group consists of four genes, *fms*, *ednrb1*, *leopard*, and *primrose*, each of which is required for development of LSMs but not ESMs (Johnson *et al.*, 1995b; Parichy *et al.*, 2000a,b; S.L.J., unpublished data). Because fish doubly mutant for one gene in each epistasis group lack virtually all body melanocytes (Fig. 1; Johnson *et al.*, 1995b; Parichy *et al.*, 2000b), these two epistasis groups represent two genetically defined classes of melanocytes that comprise the adult zebrafish body stripes. As these different melanocyte classes are defined strictly by genetic and developmental criteria, it would be useful to identify molecular markers which distinguish between the different classes.

An interesting question is how these different classes of melanocytes may have evolved. The finding that the paralogous receptor tyrosine kinases *kit* and *fms* control the development of parallel populations of body stripe melanocytes raises the possibility that additional steps in the development of these distinct melanocyte classes may be

© 2001 Elsevier Science. All rights reserved.

TABLE 2
Zebrafish Melanocyte Classes Present (+) or Absent (−) in Selected Pigment Pattern Mutants

Mutant (gene)	Embryonic melanocytes	Early stripe melanocytes	Late stripe melanocytes	Primary fin melanocytes	Secondary fin melanocytes
sparse (*kit*)	−	−	+	−	+
rose[a] (*ednrb1*)	+	+	−	+	N/A
panther (*fms*)	+	+	−	+	N/A
nacre (*mitf*)	−	−	−	−	−

[a] Mutations in *leopard* (Johnson *et al.*, 1995b) and *primrose* (S.L.J., unpublished data) also affect these cells.

regulated by other paralogous gene pairs. The finding of a third class of adult melanocytes that develops in the caudal and anal fins in the absence of *kit*-dependent and *fms*-dependent melanocytes (secondary fin regeneration melanocytes; Fig. 1; Johnson *et al.*, 1995b; Parichy *et al.*, 2000b; Rawls and Johnson, 2000) raises the possibility that another paralog to *kit* and *fms* may promote their development. Considering the possibility that the zebrafish genome experienced a partial or complete duplication (Postlethwait *et al.*, 1998; Barbazuk *et al.*, 2000), the developmental control of these distinct pigment patterns by apparently paralogous genes may provide an example of how gene or genome duplications can produce divergent developmental pathways.

Several important questions about the relationships between the multiple classes of zebrafish melanocytes remain unanswered. For instance, it is unknown whether zebrafish *kit* and *fms* bind their appropriately orthologous ligands, or whether they share a single ligand. However, zebrafish orthologs for the ligands of *kit* and *fms* remain unidentified despite directed screens and EST projects. Also, the lineage relationship between the different melanocyte populations needs to be investigated. For instance, it remains unclear whether the multiple classes of adult melanocytes arise from either a single class or multiple classes of melanocyte stem cells.

Stem Cell Regulation

Stem cells are important in the development and homeostasis of a variety of vertebrate tissues, and the recent evidence for melanocyte stem cells in both zebrafish (Johnson *et al.*, 1995b; Rawls and Johnson, 2000) and mammals (Slominski *et al.*, 1996; Kunisada *et al.*, 1998; Botchkareva *et al.*, 2001) raises several questions that might be addressed with forward genetic analysis in zebrafish. For example, what are the mechanisms that control the establishment of stem cell populations early in development, their maintenance during ontogeny, and their recruitment to produce differentiated progeny during metamorphosis, growth, and homeostasis?

To understand the mechanisms underlying the development of melanocyte stem cells throughout ontogeny, questions about the temporal requirements for genes important in melanocyte development must be addressed. For example, deficits in adult pigment pattern caused by constitutive loss of gene function could be due to requirements for that gene early in development to establish or maintain stem cell populations, or later in development to recruit stem cells to form new melanocytes. While constitutive mutations can provide some insight, dissection of temporal gene requirements necessitates the use of conditional mutations. For example, use of a tetracycline-inducible system allowed for engineering of a conditional disruption of *Ednrb* in mouse that revealed that *Ednrb* is required during a discrete period of embryonic development to promote migration of melanoblasts and presumably melanocyte stem cells, or their survival following migration (Shin *et al.*, 1999).

Questions of temporal requirement have perhaps been most thoroughly addressed for the *Kit* receptor tyrosine kinase. As described above, zebrafish *kit* mutants are deficient in stem cell-derived melanocytes that contribute to the adult body and fin stripes (Johnson *et al.*, 1995b; Rawls and Johnson, 2000). Specifically, while regenerating wild-type fins form new melanocytes beginning 4 days after amputation, *kit* mutants fail to form new cells through day 7. Since *kit* function is also required for the migration and survival of embryonic neural crest-derived melanocytes and their precursors (Motro *et al.*, 1991; Cable *et al.*, 1995; Wehrle-Haller and Weston, 1995; MacKenzie *et al.*, 1997; Parichy *et al.*, 1999; Wehrle-Haller *et al.*, 2001), it has been proposed that adult pigment pattern phenotypes in *kit* mutants might be due to requirements for *kit* during early stages of ontogeny to promote the development of adult melanocyte stem cells (Huszar *et al.*, 1991; Nishikawa *et al.*, 1991; Wehrle-Haller and Weston, 1995; Yoshida *et al.*, 1996; MacKenzie *et al.*, 1997; Rawls and Johnson, 2000). Alternatively, *kit* might be required during regeneration to recruit melanocyte stem cells to reenter developmental pathways or promote subsequent melanoblast development. In the absence of molecular markers for melanocyte stem cells, we sought to distinguish between possible roles for *kit* during early developmental stages or during regeneration, using a conditional temperature-sensitive mutation of zebrafish *kit*. Temperature-shift experiments

© 2001 Elsevier Science. All rights reserved.

showed that *kit* is required during adult fin regeneration to promote the population of the regenerate by melanoblasts, rather than during earlier developmental stages to establish or maintain their stem cell precursors (Rawls and Johnson, 2001). Adult roles for *Kit* in recruiting melanocyte stem cells to form new melanocytes in mice have also been suggested by studies using conditional abrogation of gene function with Kit-directed antibodies (Nishikawa *et al.*, 1991; Kunisada *et al.*, 1998; Botchkareva *et al.*, 2001). These studies also suggested roles for murine *Kit* early in development in establishing melanocyte stem cells (Nishikawa *et al.*, 1991; Yoshida *et al.*, 1996), indicating that there could be differences in the developmental roles of *Kit* in melanocyte stem cell development between zebrafish and mice.

In addition to facilitating the dissection of known gene requirements, conditional mutations can also reveal new ones. For example, we used the temperature-sensitive zebrafish *kit* allele to demonstrate a transient role for *kit* in promoting the survival of new regeneration melanocytes following their overt differentiation (Rawls and Johnson, 2001). While acquisition of *Kit*-independence has been suggested by studies *in vitro* (Morrison-Graham and Weston, 1993) or *in vivo* using Kit-directed antibodies (Yoshida *et al.*, 1996), our results demonstrate transition to *Kit*-independence in differentiated melanocytes as revealed by genetic manipulation. Understanding how melanocytes transit from *Kit*-dependence to growth factor- or growth factor receptor-independence will be of substantial interest.

While the ability of the zebrafish embryonic neural crest to regulate in response to genetic or physical cell ablation is well-recognized (Milos and Dingle, 1978b; Raible and Eisen, 1996; Vaglia and Hall, 2000; reviewed in Vaglia and Hall, 1999), the regulative capacity of melanocyte progenitors during adult stages is only beginning to be appreciated. A striking example is found in the ability of stem cell-derived secondary fin regeneration melanocytes to completely reestablish the regenerating fin stripe in the absence of *kit* function and the ensuing absence of primary fin regeneration melanocytes. This secondary (*kit*-independent) class of fin regeneration melanocytes has a remarkable capacity for regulation, as it eventually reestablishes the entire fin stripe at wild-type melanocyte densities (Rawls and Johnson, 2000). This contrasts sharply with *kit*-dependent (ESM) and *fms*-dependent (LSM) melanocytes in the adult body stripe, each of which contributes only half the normal number of melanocytes in the absence of the other population (Johnson *et al.*, 1995b; Parichy *et al.*, 2000b). Although secondary fin regeneration melanocytes have little or no role in normal fin stripe regeneration, they may fill holes in the stripe during late stages of wild-type regeneration (Rawls and Johnson, 2000). The striking regulative capacity of secondary fin regeneration melanocytes and their stem cell precursors in the fin provides a unique opportunity for forward genetic analysis of stem cell regulation.

Simple Models of Organogenesis

Perhaps the aspect of pigment cell biology that has fascinated people more than any other is the variety of pigment patterns found either in closely related species or even within a single species. Despite this long-standing interest, we still know remarkably little about how different pigment patterns are generated. Vertebrates employ two general strategies for generating different pigment patterns, that we broadly refer to here as physiological and morphological patterning. In physiological control of pigment pattern, pigment cells can change the type of pigment that they produce, or redistribute pigment granules within the cell. Striking examples of physiological control are found in the rapid color changes of the chameleon and the cuttlefish (Holmes, 1940; Loi *et al.*, 1996). Although pigment cell physiology might play a relatively smaller role in zebrafish pigment patterning, fish can rapidly and reversibly alter the intracellular localization of pigment granules to create different pigment pattern appearances (McNiven and Porter, 1984; Jimbow and Sugiyama, 1998). Less dynamic but more familiar examples of physiological control of pigment pattern are the phenotypes of the *recessive yellow* and *agouti* mouse coat color mutants, which are caused by shifts in the type of melanin deposited in growing hairs (Silvers, 1979; Vrieling *et al.*, 1994; reviewed in Barsh, 1996). Notably, melanocyte-stimulating hormone signaling plays important roles in these processes in both poikilotherms and mammals (reviewed in Barsh, 1996; Bagnara, 1998).

A second strategy for generating different pigment patterns is through morphological control, made possible by affecting the localization of pigment cells within the animal. The organization of pigment cells into different patterns is of particular interest because it provides a simple model for organogenesis in which the component cells are not essential for viability. In mammals, morphological control might be demonstrated in the restricted localization of melanocytes found in white-spotting (Silvers, 1956) or piebald patterning in mice (Mayer, 1965; Silvers, 1979). In zebrafish, this strategy is evident in the wild-type adult stripe pattern, and is dramatically disrupted in stripe pattern mutants, such as *jaguar* that has broader stripes (Johnson *et al.*, 1995a), *leopard* that has spots (Fig. 2; Kirschbaum, 1975; Johnson *et al.*, 1995b), and *fms* (*panther*) that has mostly dispersed melanocytes (Fig. 1; Parichy *et al.*, 2000b). An outstanding question is whether physiological or morphological mechanisms as described here are responsible for producing such familiar vertebrate pigment patterns as the stripes of the zebra and the spots of the leopard. A more thorough understanding of pigment patterning in model organisms will lay the foundation for understanding patterning mechanisms in other animals.

While different mathematical models have been proposed to account for complex pigment patterns in vertebrates (Murray, 1981, 1989; Nagorcka and Mooney, 1992; Koch and Meinhardt, 1994), acquiring molecular evidence for

© 2001 Elsevier Science. All rights reserved.

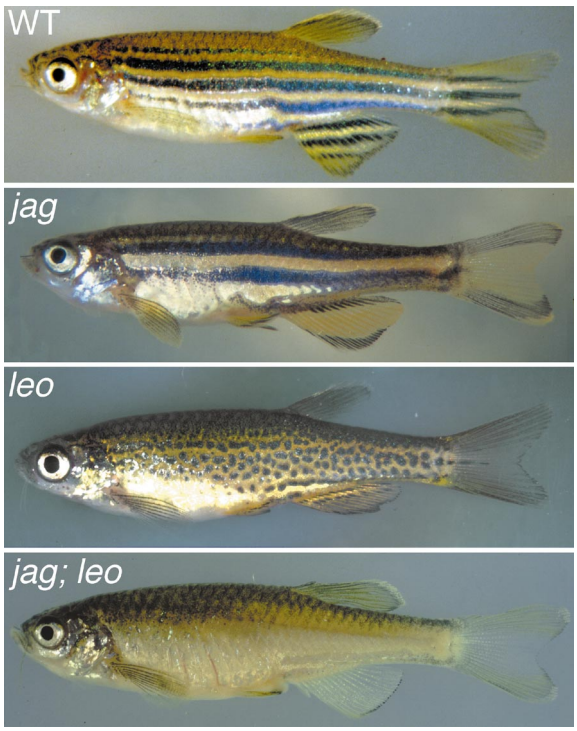

FIG. 2. Zebrafish stripe mutants cause distinct patterning defects. Adult wild-type zebrafish (WT) have several stripes in the body and fins. Homozygous *jaguar*^{b230} mutants (*jag*) have fewer and broader stripes, while homozygous *leopard*^{t1} mutants (*leo*) have spots. Fish homozygous for both *jag*^{b230} and *leo*^{t1} (*jag;leo*) lack all body stripe melanocytes, but retain dorsal scale-associated melanocytes.

these hypothetical mechanisms has been slow. The pigment pattern mutants of zebrafish may provide this necessary molecular evidence. Several major classes of zebrafish pigment pattern phenotypes have emerged, including broad stripe mutants like *jaguar* (Johnson *et al.*, 1995a) and spotting mutants like *leopard* (Fig. 2; Kirschbaum, 1975; Johnson *et al.*, 1995b). Asai and coworkers (1999) showed that an allelic series of the *leopard* mutant can be modeled by reaction-diffusion equations, suggesting that identification of the *leopard* gene will provide insight into how reaction-diffusion mechanisms use molecules to elicit their effects. However, mathematical models have not yet been used to explain the phenotypes of other zebrafish pigment pattern mutants, such as the *jaguar* mutant with its broader and fewer stripes. The fact that *jaguar;leopard* double mutants lack virtually all body melanocytes shows that the mechanism responsible for the *jaguar* phenotype interacts in some yet unknown way with the mechanism responsible for the *leopard* phenotype (Fig. 2). A comprehensive mathematical model for zebrafish stripe pattern formation will need to account for *leopard*, *jaguar*, and their double mutant phenotypes, as well as other patterns that have been described.

It is useful to think of the pigment pattern as a simple organ system. A complete understanding of organogenesis should include knowledge of how different cells interact to assemble the desired structure. Therefore, when considering the mechanisms of adult melanocyte stripe formation in zebrafish, interactions and relationships between melanocytes and the other types of pigment cells must be taken into account. Genetic analysis suggests a hierarchical model of interactions between different types of pigment cells in zebrafish. For example, melanocytes direct the patterning of iridophores in the adult body stripes (Johnson *et al.*, 1995b), while normal migration and survival of adult stripe melanocytes are in turn directed in part by xanthophores (Parichy *et al.*, 2000b). The observation that adult melanocytes which form in the absence of xanthophores in *fms* mutants are able to partially aggregate into stripes suggests that melanocyte patterning does not depend entirely on xanthophores. Identification of the genes affected in zebrafish stripe pattern mutants will extend our understanding of these processes.

FUTURE DIRECTIONS

While study of melanocyte development in zebrafish and mammals has revealed substantial differences in mechanism and gene requirement, an apparently high degree of similarity has emerged due in part to the successful use of mouse genes as candidates for zebrafish mutants. However, mutant screens have already produced many zebrafish pigment pattern mutants (Johnson and Weston, 1995; Driever *et al.*, 1996; Henion *et al.*, 1996; Odenthal *et al.*, 1996; Kelsh *et al.*, 1996, 2000) which are still in the process of being characterized. The abundance of zebrafish pigment pattern mutants and the differences in developmental strategy between mammalian and zebrafish pigment patterns suggest that corroborative findings will become increasingly less common. Indeed, unexpected differences between zebrafish and mammalian pigment pattern development have already been revealed in their respective requirements for *fms* (Parichy *et al.*, 2000b).

In the same way that the inherent labeling of differentiated pigment cells has been successfully used to identify pigment pattern mutants, the advent of transgenic technology in zebrafish using cell-specific expression of green fluorescent protein or other markers (Luo *et al.*, 2001) will allow visualization of pigment cell precursors *in vivo*. New generations of mutant screens using these reagents will undoubtedly expand the available panel of mutants and extend our understanding of the development of vertebrate pigment cells and other neural crest derivatives. Since the speed of mapping and cloning zebrafish mutations will be greatly accelerated by the ongoing sequencing of the zebrafish genome and the growth of genomic resources (Geisler *et al.*, 1999; Barbazuk *et al.*, 2000), zebrafish will remain one of the preferred vertebrate model systems for

© 2001 Elsevier Science. All rights reserved.

pigment pattern morphogenesis and other aspects of developmental biology.

ACKNOWLEDGMENTS

We thank Douglas Creer and Susan Dutcher for their helpful criticism of this manuscript. This work was supported by NIH Grant R01-GM56988 and a Pew Scholars Award (to S.L.J.).

REFERENCES

Attie, T., Till, M., Pelet, A., Amiel, J., Edery, P., Boutrand, L., Munnich, A., and Lyonnet, S. (1995). Mutation of the endothelin-receptor B gene in Waardenburg–Hirschsprung disease. *Hum. Mol. Genet.* **4,** 2407–2409.

Bagnara, J. T. (1998). Comparative anatomy and physiology of pigment cells in nonmammalian tissues. In "The Pigmentary System: Physiology and Pathophysiology" (J. J. Nordlund, R. E. Boissy, V. J. Hearing, R. A. King, and J.-P. Ortonne, Eds.), pp. 9–40. Oxford Univ. Press, New York.

Barbazuk, W. B., Korf, I., Kadavi, C., Heyen, J., Tate, S., Wun, E., Bedell, J. A., McPherson, J. D., and Johnson, S. L. (2000). The syntenic relationship of the zebrafish and human genomes. *Genome Res.* **10,** 1351–1358.

Barsh, G. S. (1996). The genetics of pigmentation: From fancy genes to complex traits. *Trends Genet.* **12,** 299–305.

Baynash, A. G., Hosoda, K., Giaid, A., Richardson, J. A., Emoto, N., Hammer, R. E., and Yanagisawa, M. (1994). Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. *Cell* **79,** 1277–1285.

Bentley, N. J., Eisen, T., and Goding, C. R. (1994). Melanocyte-specific expression of the human tyrosinase promoter: Activation by the microphthalmia gene product and role of the initiator. *Mol. Cell. Biol.* **14,** 7996–8006.

Bernex, F., De Sepulveda, P., Kress, C., Elbaz, C., Delouis, C., and Panthier, J. J. (1996). Spatial and temporal patterns of c-*kit*-expressing cells in $W^{lacZ}/+$ and W^{lacZ}/W^{lacZ} mouse embryos. *Development* **122**, 3023–3033.

Bertolotto, C., Abbe, P., Hemesath, T. J., Bille, K., Fisher, D. E., Ortonne, J. P., and Ballotti, R. (1998). Microphthalmia gene product as a signal transducer in cAMP-induced differentiation of melanocytes. *J. Cell Biol.* **142,** 827–835.

Besmer, P., Manova, K., Duttlinger, R., Huang, E. J., Packer, A., Gyssler, C., and Bachvarova, R. F. (1993). The *kit*-ligand (steel factor) and its receptor c-*kit*/*W*: Pleiotropic roles in gametogenesis and melanogenesis. *Dev. Suppl.*, 125–137.

Bondurand, N., Pingault, V., Goerich, D. E., Lemort, N., Sock, E., Caignec, C. L., Wegner, M., and Goossens, M. (2000). Interaction among SOX10, PAX3 and MITF, three genes altered in Waardenburg syndrome. *Hum. Mol. Genet.* **9,** 1907–1917.

Botchkareva, N. V., Khlgatian, M., Longley, B. J., Botchkarev, V. A., and Gilchrest, B. A. (2001). SCF/c-kit signaling is required for cyclic regeneration of the hair pigmentation unit. *FASEB J.* **15,** 645–658.

Britsch, S., Goerich, D. E., Riethmacher, D., Peirano, R. I., Rossner, M., Nave, K. A., Birchmeier, C., and Wegner, M. (2001). The transcription factor Sox10 is a key regulator of peripheral glial development. *Genes Dev.* **15,** 66–78.

Broudy, V. C. (1997). Stem cell factor and hematopoiesis. *Blood* **90,** 1345–1364.

Busca, R., and Ballotti, R. (2000). Cyclic AMP a key messenger in the regulation of skin pigmentation. *Pigment Cell Res.* **13,** 60–69.

Cable, J., Jackson, I. J., and Steel, K. P. (1995). Mutations at the *W* locus affect survival of neural crest-derived melanocytes in the mouse. *Mech. Dev.* **50,** 139–150.

Castle, W. E., and Little, C. C. (1910). On a modified Mendelian ratio among yellow mice. *Science* **32,** 868–870.

Chakravarti, A. (1996). Endothelin receptor-mediated signaling in Hirschsprung disease. *Hum. Mol. Genet.* **5,** 303–307.

Christiansen, J. H., Coles, E. G., and Wilkinson, D. G. (2000). Molecular control of neural crest formation, migration and differentiation. *Curr. Opin. Cell Biol.* **12,** 719–724.

Cuénot, L. (1908). Sur quelques anomalies apparentes des proportions Mendeliennes. *Notes Renne* **Ib, 9,** 7–15.

Dorsky, R. I., Moon, R. T., and Raible, D. W. (1998). Control of neural crest cell fate by the Wnt signalling pathway. *Nature* **396,** 370–373.

Dorsky, R. I., Moon, R. T., and Raible, D. W. (2000a). Environmental signals and cell fate specification in premigratory neural crest. *BioEssays* **22,** 708–716.

Dorsky, R. I., Raible, D. W., and Moon, R. T. (2000b). Direct regulation of *nacre*, a zebrafish MITF homolog required for pigment cell formation, by the Wnt pathway. *Genes Dev.* **14,** 158–162.

Driever, W., Solnica-Krezel, L., Schier, A. F., Neuhauss, S. C., Malicki, J., Stemple, D. L., Stainier, D. Y., Zwartkruis, F., Abdelilah, S., Rangini, Z., Belak, J., and Boggs, C. (1996). A genetic screen for mutations affecting embryogenesis in zebrafish. *Development* **123,** 37–46.

Dunn, K. J., Williams, B. O., Li, Y., and Pavan, W. J. (2000). Neural crest-directed gene transfer demonstrates Wnt1 role in melanocyte expansion and differentiation during mouse development. *Proc. Natl. Acad. Sci. USA* **97,** 10050–10055.

Dupin, E., Glavieux, C., Vaigot, P., and Le Douarin, N. M. (2000). Endothelin 3 induces the reversion of melanocytes to glia through a neural crest-derived glial-melanocytic progenitor. *Proc. Natl. Acad. Sci. USA* **97,** 7882–7887.

Dutton, K. A., Pauliny, A., Lopes, S. S., Elworthy, S., Carney, T. J., Rauch, J., Geisler, R., Haffter, P., and Kelsh, R. N. (2001). Zebrafish *colourless* encodes *sox10* and specifies non-ectomesenchymal neural crest fates. *Development* **128,** in press.

Edery, P., Attie, T., Amiel, J., Pelet, A., Eng, C., Hofstra, R. M., Martelli, H., Bidaud, C., Munnich, A., and Lyonnet, S. (1996). Mutation of the endothelin-3 gene in the Waardenburg–Hirschsprung disease (Shah–Waardenburg syndrome). *Nat. Genet.* **12,** 442–444.

Epstein, D. J., Vekemans, M., and Gros, P. (1991). *Splotch* (*Sp2H*), a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of Pax-3. *Cell* **67,** 767–774.

Felix, R., Hofstetter, W., Wetterwald, A., Cecchini, M. G., and Fleisch, H. (1994). Role of colony-stimulating factor-1 in bone metabolism. *J. Cell. Biochem.* **55,** 340–349.

Fisher, D. E. (2000). Microphthalmia: A signal responsive transcriptional regulator in development. *Pigment Cell Res.* **13**(Suppl. 8), 145–149.

Flanagan, A. M., and Lader, C. S. (1998). Update on the biologic effects of macrophage colony-stimulating factor. *Curr. Opin. Hematol.* **5,** 181–185.

Geisler, R., Rauch, G. J., Baier, H., van Bebber, F., Brobeta, L., Dekens, M. P., Finger, K., Fricke, C., Gates, M. A., Geiger, H.,

© 2001 Elsevier Science. All rights reserved.

Geiger-Rudolph, S., Gilmour, D., Glaser, S., Gnugge, L., Habeck, H., Hingst, K., Holley, S., Keenan, J., Kirn, A., Knaut, H., Lashkari, D., Maderspacher, F., Martyn, U., Neuhauss, S., Haffter P, *et al.* (1999). A radiation hybrid map of the zebrafish genome. *Nat. Genet.* **23,** 86–89.

Geissler, E. N., Ryan, M. A., and Housman, D. E. (1988). The dominant-white spotting (*W*) locus of the mouse encodes the c-*kit* proto-oncogene. *Cell* **55,** 185–192.

Giebel, L. B., and Spritz, R. A. (1991). Mutation of the *KIT* (mast/stem cell growth factor receptor) protooncogene in human piebaldism. *Proc. Natl. Acad. Sci. USA* **88,** 8696–8699.

Goding, C. R. (2000). Mitf from neural crest to melanoma: Signal transduction and transcription in the melanocyte lineage. *Genes Dev.* **14,** 1712–1728.

Goodrich, H. B., and Nichols, R. (1931). The development and the regeneration of the color pattern in *Brachydanio rerio*. *J. Morphol.* **52,** 513–523.

Groves, A. K., and Bronner-Fraser, M. (1999). Neural crest diversification. *Curr. Top. Dev. Biol.* **43,** 221–258.

Hallsson, J. H., Favor, J., Hodgkinson, C., Glaser, T., Lamoreux, M. L., Magnusdottir, R., Gunnarsson, G. J., Sweet, H. O., Copeland, N. G., Jenkins, N. A., and Steingrimsson, E. (2000). Genomic, transcriptional and mutational analysis of the mouse *microphthalmia* locus. *Genetics* **155,** 291–300.

Hemesath, T. J., Price, E. R., Takemoto, C., Badalian, T., and Fisher D. E. (1998). MAP kinase links the transcription factor Microphthalmia to c-Kit signalling in melanocytes. *Nature* **391,** 298–301.

Hemesath, T. J., Steingrimsson, E., McGill, G., Hansen, M. J., Vaught, J., Hodgkinson, C. A., Arnheiter, H., Copeland, N. G., Jenkins, N. A., and Fisher, D. E. (1994). Microphthalmia, a critical factor in melanocyte development, defines a discrete transcription factor family. *Genes Dev.* **8,** 2770–2780.

Henion, P. D., Raible, D. W., Beattie, C. E., Stoesser, K. L., Weston, J. A., and Eisen, J. S. (1996). Screen for mutations affecting development of Zebrafish neural crest. *Dev. Genet.* **18,** 11–17.

Hodgkinson, C. A., Moore, K. J., Nakayama, A., Steingrimsson, E., Copeland, N. G., Jenkins, N. A., and Arnheiter, H. (1993). Mutations at the mouse microphthalmia locus are associated with defects in a gene encoding a novel basic-helix–loop–helix-zipper protein. *Cell* **74,** 395–404.

Hofstra, R. M., Osinga, J., Tan-Sindhunata, G., Wu, Y., Kamsteeg, E. J., Stulp, R. P., van Ravenswaaij-Arts, C., Majoor-Krakauer, D., Angrist, M., Chakravarti, A., Meijers, C., and Buys, C. H. (1996). A homozygous mutation in the endothelin-3 gene associated with a combined Waardenburg type 2 and Hirschsprung phenotype (Shah–Waardenburg syndrome). *Nat. Genet.* **12,** 445–447.

Holmes, W. (1940). The color changes and colour patterns of *Sepia officinalis*. L. *Proc. Zool. Soc.* **110,** 17–35.

Hornyak, T. J., Hayes, D. J., Chiu, L., and Ziff, E. B. (2001). Transcription factors in melanocyte development: Distinct roles for Pax-3 and Mitf. *Mech. Dev.* **101,** 47–59.

Horstadius, S. (1950). "The Neural Crest: Its Properties and Derivatives in the Light of Experimental Research." Oxford Univ. Press, London.

Hosoda, K., Hammer, R. E., Richardson, J. A., Baynash, A. G., Cheung, J. C., Giaid, A., and Yanagisawa, M. (1994). Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell* **79,** 1267–1276.

Hoth, C. F., Milunsky, A., Lipsky, N., Sheffer, R., Clarren, S. K., and Baldwin, C. T. (1993). Mutations in the paired domain of the human PAX3 gene cause Klein–Waardenburg syndrome (WS-III) as well as Waardenburg syndrome type I (WS-I). *Am. J. Hum. Genet.* **52,** 455–462.

Hou, L., Panthier, J. J., and Arnheiter, H. (2000). Signaling and transcriptional regulation in the neural crest-derived melanocyte lineage: Interactions between KIT and MITF. *Development* **127,** 5379–5389.

Huszar, D., Sharpe, A., and Jaenisch, R. (1991). Migration and proliferation of cultured neural crest cells in *W* mutant neural crest chimeras. *Development* **112**, 131–141.

Ikeya, M., Lee, S. M., Johnson, J. E., McMahon, A. P., and Takada, S. (1997). Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* **389,** 966–970.

Inoue, K., Tanabe, Y., and Lupski, J. R. (1999). Myelin deficiencies in both the central and the peripheral nervous systems associated with a SOX10 mutation. *Ann. Neurol.* **46,** 313–318.

Ito, M., Kawa, Y., Ono, H., Okura, M., Baba, T., Kubota, Y., Nishikawa, S. I., and Mizoguchi, M. (1999). Removal of stem cell factor or addition of monoclonal anti-c-KIT antibody induces apoptosis in murine melanocyte precursors. *J. Invest. Dermatol.* **112,** 796–801.

Jimbow, K., and Sugiyama, S. (1998). Melanosomal translocation and transfer. In "The Pigmentary System: Physiology and Pathophysiology" (J. J. Nordlund, R. E. Boissy, V. J. Hearing, R. A. King, and J.-P. Ortonne, Eds.), pp. 107–114. Oxford Univ. Press, New York.

Jin, E. J., Erickson, C. A., Takada, S., and Burrus, L. W. (2001). Wnt, and BMP signaling govern lineage segregation of melanocytes in the avian embryo. *Dev. Biol.* **233,** 22–37.

Johnson, S. L., Africa, D., Horne, S., and Postlethwait, J. H. (1995a). Half-tetrad analysis in zebrafish: Mapping the *ros* mutation and the centromere of linkage group I. *Genetics* **139,** 1727–1735.

Johnson, S. L., Africa, D., Walker, C., and Weston, J. A. (1995b). Genetic control of adult pigment stripe development in zebrafish. *Dev. Biol.* **167,** 27–33.

Johnson, S. L., and Bennett, P. (1999). Growth control in the ontogenetic and regenerating zebrafish fin. *Methods Cell Biol.* **59,** 301–311.

Johnson, S. L., and Weston, J. A. (1995). Temperature-sensitive mutations that cause stage specific defects in zebrafish fin regeneration. *Genetics* **141,** 1588–1595.

Jordan, S. A., and Jackson, I. J. (2000). MGF (KIT ligand) is a chemokinetic factor for melanoblast migration into hair follicles. *Dev. Biol.* **225,** 424–436.

Kelsh, R. N., Brand, M., Jiang, Y. J., Heisenberg, C. P., Lin, S., Haffter, P., Odenthal, J., Mullins, M. C., van Eeden, F. J., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Kane, D. A., Warga, R. M., Beuchle, D., Vogelsang, L., and Nusslein-Volhard, C. (1996). Zebrafish pigmentation mutations and the processes of neural crest development. *Development* **123,** 369–389.

Kelsh, R. N., and Eisen, J. S. (2000). The zebrafish *colourless* gene regulates development of non-ectomesenchymal neural crest derivatives. *Development* **127,** 515–525.

Kelsh, R. N., Schmid, B., and Eisen, J. S. (2000). Genetic analysis of melanophore development in zebrafish embryos. *Dev. Biol.* **225,** 277–293.

Kirschbaum, F. (1975). Untersuchungen über das Farbmuster der Zebrabarbe *Brachydanio rerio* (Cyprinidae, Teleostei). *Roux's Arch. Dev. Biol.* **177,** 129–152.

Koch, A. J., and Meinhardt, H. (1994). Biological pattern formation: From basic mechanism to complex structures. *Rev. Mod. Phys.* **66,** 1481–1507.

© 2001 Elsevier Science. All rights reserved.

Kunisada, T., Yoshida, H., Yamazaki, H., Miyamoto, A., Hemmi, H., Nishimura, E., Shultz, L. D., Nishikawa, S., and Hayashi, S. (1998). Transgene expression of steel factor in the basal layer of epidermis promotes survival, proliferation, differentiation and migration of melanocyte precursors. *Development* **125,** 2915–2923.

Lahav, R., Dupin, E., Lecoin, L., Glavieux, C., Champeval, D., Ziller, C., and Le Douarin, N. M. (1998). Endothelin 3 selectively promotes survival and proliferation of neural crest-derived glial and melanocytic precursors in vitro. *Proc. Natl. Acad. Sci. USA* **95,** 14214–14219.

Le Douarin, N. (1982). "The Neural Crest." Cambridge Univ. Press, Cambridge, UK.

Lee, M., Goodall, J., Verastegui, C., Ballotti, R., Goding, C. R. (2000). Direct regulation of the *Microphthalmia* promoter by Sox10 links Waardenburg-Shah syndrome (WS4)-associated hypopigmentation and deafness to WS2. *J. Biol. Chem*. **275,** 37978–37983.

Lerner, A. B., Shiohara, T., Boissy, R. E., Jacobson, K. A., Lamoreux, M. L., and Moellmann, G. E. (1986). A mouse model for vitiligo. *J. Invest. Dermatol.* **87,** 299–304.

Linnekin, D. (1999). Early signaling pathways activated by c-Kit in hematopoietic cells. *Int. J. Biochem. Cell Biol.* **31,** 1053–1074.

Lister, J. A., Close, J., and Raible, D. W. (2001). Duplicate *mitf* genes in zebrafish: Complementary expression and conservation of melanogenic potential. *Dev. Biol.* **237,** 333–344.

Lister, J. A., Robertson, C. P., Lepage, T., Johnson, S. L., and Raible, D. W. (1999). *nacre* encodes a zebrafish microphthalmia-related protein that regulates neural-crest-derived pigment cell fate. *Development* **126,** 3757–3767.

Loi, P., Saunders, R., Young, D, and Tublitz, N. (1996). Peptidergic regulation of chromatophore function in the European cuttlefish *Sepia officinalis. J. Exp. Biol.* **199,** 1177–1187.

Luo, R., An, M., Arduini, B. L., and Henion, P. D. (2001). Specific pan-neural crest expression of zebrafish *Crestin* throughout embryonic development. *Dev. Dyn.* **220,** 169–174.

MacKenzie, M. A. F., Jordan, S. A., Budd, P. S., and Jackson, I. J. (1997). Activation of the receptor tyrosine kinase Kit is required for the proliferation of melanoblasts in the mouse embryo. *Dev. Biol.* **192,** 99–107.

Marklund, S., Kijas, J., Rodriguez-Martinez, H., Ronnstrand, L., Funa, K., Moller, M., Lange, D., Edfors-Lilja, I., and Andersson, L. (1998). Molecular basis for the dominant white phenotype in the domestic pig. *Genome Res.* **8,** 826–833.

Marks, S. C., Jr., and Lane, P. W. (1976). *Osteopetrosis*, a new recessive skeletal mutation on chromosome 12 of the mouse. *J. Hered.* **67,** 11–18.

Mayer, T. C. (1965). The development of piebald spotting in mice. *Dev. Biol.* **11,** 319–334.

McMahon, A. P., and Bradley, A. (1990). The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain. *Cell* **62,** 1073–1085.

McNiven, M. A., and Porter, K. R. (1984). Chromatophores: Models for studying cytomatrix translocations. *J. Cell Biol.* **99** 152s–158s.

Milos, N., and Dingle, A. D. (1978a). Dynamics of pigment pattern formation in the zebrafish, *Brachydanio rerio*. I. Establishment and regulation of the lateral line melanophore stripe during the first eight days of development. *J. Exp. Zool.* **205,** 205–216.

Milos, N., and Dingle, A. D. (1978b). Dynamics of pigment pattern formation in the zebrafish, *Brachydanio rerio*. II. Lability of lateral line strip formation and regulation of pattern defects. *J. Exp. Zool.* **205,** 217–224.

Mintz, B, and Russell, E. S. (1957). Gene-induced embryological modifications of primordial germ cells in the mouse. *J. Exp. Zool.* **134,** 207–237.

Moore, K. J. (1995). Insight into the *microphthalmia* gene. *Trends Genet.* **11,** 442–428.

Morrison-Graham, K., and Weston, J. A. (1993). Transient steel factor dependence by neural crest-derived melanocyte precursors. *Dev. Biol.* **159,** 346–352.

Motoyoshi, K. (1998). Biological activities and clinical application of M-CSF. *Int. J. Hematol.* **67,** 109–122.

Motro, B., van der Kooy, D., Rossant, J., Reith, A., and Bernstein, A. (1991). Contiguous patterns of c-*kit* and *steel* expression: Analysis of mutations at the *W* and *Sl* loci. *Development* **113,** 1207–1221.

Murray, J. D. (1981). On pattern formation mechanisms for lepidopteran wing patterns and mammalian coat markings. *Philos. Trans. R. Soc. London B* **295,** 473–496.

Murray, J. D. (1989). "Mathematical Biology." Springer-Verlag, New York.

Nagorcka, B. N., and Mooney, J. R. (1992). From stripes to spots: prepatterns which can be produced in the skin by a reaction-diffusion system. *IMA J. Math. Appl. Med. Biol.* **9,** 249–267.

Nishikawa, S., Kusakabe, M., Yoshinaga, K., Ogawa, M., Hayashi, S., Kunisada, T., Era, T., Sakakura, T., and Nishikawa, S. (1991). In utero manipulation of coat color formation by a monoclonal anti-c-*kit* antibody: Two distinct waves of c-*kit*-dependency during melanocyte development. *EMBO J.* **10**, 2111–2118.

Nocka, K., Tan, J. C., Chiu, E., Chu, T. Y., Ray, P., Traktman, P., and Besmer, P. (1990). Molecular bases of dominant negative and loss of function mutations at the murine c-*kit*/*white spotting* locus: W^{37}, W^{v}, W^{41} and *W*. *EMBO J.* **9,** 1805–1813.

Nordlund, J. J., Boissy, R. E., Hearing, V. J., King, R. A., and Ortonne, J.-P. (1998). "The Pigmentary System: Physiology and Pathophysiology." Oxford Univ. Press, New York.

Odenthal, J., Rossnagel, K., Haffter, P., Kelsh, R. N., Vogelsang, E., Brand, M., van Eeden, F. J., Furutani-Seiki, M., Granato, M., Hammerschmidt, M., Heisenberg, C. P., Jiang, Y. J., Kane, D. A., Mullins, M. C., and Nusslein-Volhard, C. (1996). Mutations affecting xanthophore pigmentation in the zebrafish *Danio rerio*. *Development* **123,** 391–398.

Opdecamp, K., Nakayama, A., Nguyen, M. T., Hodgkinson, C. A., Pavan, W. J., and Arnheiter, H. (1997). Melanocyte development in vivo and in neural crest cell cultures: Crucial dependence on the Mitf basic-helix–loop–helix-zipper transcription factor. *Development* **124,** 2377–2386.

Orr-Urtreger, A., Avivi, A., Zimmer, Y., Givol, D., Yarden, Y., and Lonai, P. (1990). Developmental expression of c-*kit*, a proto-oncogene encoded by the *W* locus. *Development* **109,** 911–923.

Parichy, D. M., and Johnson, S. L. (2001). Zebrafish hybrids suggest genetic mechanisms for pigment pattern diversification in *Danio*. *Dev. Genes Evol.* **211,** 319–328.

Parichy, D. M., Rawls, J. F., Pratt, S. J., Whitfield, T. T., and Johnson, S. L. (1999). Zebrafish *sparse* corresponds to an orthologue of c-*kit* and is required for the morphogenesis of a subpopulation of melanocytes, but is not essential for hematopoeisis or primordial germ cell development. *Development* **126,** 3425–3436.

Parichy, D. M., Mellgren, E. M., Rawls, J. F., Lopes, S. S., Kelsh, R. N., and Johnson, S. L. (2000a). Mutational analysis of *endothelin receptor b1* (*rose*) during neural crest and pigment pattern

© 2001 Elsevier Science. All rights reserved.

development in the zebrafish *Danio rerio*. *Dev. Biol.* **227,** 294–306.

Parichy, D. M., Ransom, D. G., Paw, B., Zon, L. I., and Johnson, S. L. (2000b). An ortholog of the *kit*-related gene *fms* is required for development of neural crest-derived xanthophores and a subpopulation of adult melanocytes in the zebrafish *Danio rerio*. *Development* **127,** 3031–3044.

Pingault, V., Bondurand, N., Kuhlbrodt, K., Goerich, D. E., Prehu, M. O., Puliti, A., Herbarth, B., Hermans-Borgmeyer, I., Legius, E., Matthijs, G., *et al.* (1998). SOX10 mutations in patients with Waardenburg–Hirschsprung disease. *Nat. Genet.* **18,** 171–173.

Pingault, V., Guiochon-Mantel, A., Bondurand, N., Faure, C., Lacroix, C., Lyonnet, S., Goossens, M., and Landrieu, P. (2000). Peripheral neuropathy with hypomyelination, chronic intestinal pseudo-obstruction and deafness: A developmental "neural crest syndrome" related to a SOX10 mutation. *Ann. Neurol.* **48,** 671–676.

Postlethwait, J. H., Yan, Y. L., Gates, M. A., Horne, S., Amores, A., Brownlie, A., Donovan, A., Egan, E. S., Force, A., Gong, Z., Goutel, C., Fritz, A., Kelsh, R., Knapik, E., Liao, E., Paw, B., Ransom, D., Singer, A., Thomson, M., Abduljabbar, T. S., Yelick, P., Beier, D., Joly, J. S., Larhammar, D., Rosa, F., *et al.* (1998). Vertebrate genome evolution and the zebrafish gene map. *Nat. Genet.* **18,** 345–349.

Potterf, S. B., Furumura, M., Dunn, K. J., Arnheiter, H., and Pavan, W. J. (2000). Transcription factor hierarchy in Waardenburg syndrome: Regulation of MITF expression by SOX10 and PAX3. *Hum. Genet.* **107,** 1–6.

Price, E. R., Ding, H. F., Badalian, T., Bhattacharya, S., Takemoto, C., Yao, T. P., Hemesath, T. J., and Fisher, D. E. (1998). Lineage-specific signaling in melanocytes: c-Kit stimulation recruits p300/CBP to microphthalmia. *J. Biol. Chem.* **273,** 17983–17986.

Puffenberger, E. G., Hosoda, K., Washington, S. S., Nakao, K., deWit, D., Yanagisawa, M., and Chakravarti, A. (1994). A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung's disease. *Cell* **79,** 1257–1266.

Quevedo, W. C., Jr., and Holstein, T. J. (1998). General biology of mammalian pigmentation. In "The Pigmentary System: Physiology and Pathophysiology" (J. J. Nordlund, R. E. Boissy, V. J. Hearing, R. A. King, and J.-P. Ortonne, Eds.), pp. 43–58. Oxford Univ. Press, New York.

Raible, D. W., and Eisen, J. S. (1994). Restriction of neural crest cell fate in the trunk of the embryonic zebrafish. *Development* **120,** 495–503.

Raible, D. W., and Eisen, J. S. (1996). Regulative interactions in zebrafish neural crest. *Development* **122,** 501–507.

Raible, D. W., Wood, A., Hodsdon, W., Henion, P. D., Weston, J. A., and Eisen, J. S. (1992). Segregation and early dispersal of neural crest cells in the embryonic zebrafish. *Dev. Dyn.* **195,** 29–42.

Rawls, J. F., and Johnson, S. L. (2000). Zebrafish *kit* mutation reveals primary and secondary regulation of melanocyte development during fin stripe regeneration. *Development* **127,** 3715–3724.

Rawls, J. F., and Johnson, S. L. (2001). Requirements for the *kit* receptor tyrosine kinase during regeneration of zebrafish fin melanocytes. *Development* **128,** 1943–1949.

Reid, K., Turnley, A. M., Maxwell, G. D., Kurihara, Y., Kurihara, H., Bartlett, P. F., and Murphy, M. (1996). Multiple roles for endothelin in melanocyte development: Regulation of progenitor number and stimulation of differentiation. *Development* **122,** 3911–3919.

Russell, E. S. (1979). Hereditary anemias of the mouse: A review for geneticists. *Adv. Genet.* **20,** 357–459.

Saint-Jeannet, J. P., He, X., Varmus, H. E., and Dawid, I. B. (1997). Regulation of dorsal fate in the neuraxis by Wnt-1 and Wnt-3a. *Proc. Natl. Acad. Sci. USA* **94,** 13713–13718.

Sato, S., Roberts, K., Gambino, G., Cook, A., Kouzarides, T., Goding, C. R. (1997). CBP/p300 as a co-factor for the Microphthalmia transcription factor. *Oncogene* **14,** 3083–3092.

Serbedzija, G. N., and McMahon, A. P. (1997). Analysis of neural crest cell migration in Splotch mice using a neural crest-specific LacZ reporter. *Dev. Biol.* **185,** 139–147.

Sette, C., Dolci, S., Geremia, R., and Rossi, P. (2000). The role of stem cell factor and of alternative c-*kit* gene products in the establishment, maintenance and function of germ cells. *Int. J. Dev. Biol.* **44,** 599–608.

Shibahara, S., Yasumoto, K., Amae, S., Udono, T., Watanabe, K., Saito, H., and Takeda, K. (2000). Regulation of pigment cell-specific gene expression by MITF. *Pigment Cell Res.* **13**(Suppl. 8), 98–102.

Shin, M. K., Levorse, J. M., Ingram, R. S., and Tilghman, S. M. (1999). The temporal requirement for endothelin receptor-B signalling during neural crest development. *Nature* **402,** 496–501.

Silver, L. M. (1995). "Mouse Genetics: Concepts and Applications." Oxford Univ. Press, London.

Silvers, W. K. (1956). Pigment cells: Occurrence in hair follicles. *J. Morphol.* **99,** 41–56.

Silvers, W. K. (1979). "The Coat Colors of Mice. A Model for Mammalian Gene Action and Interaction." Springer-Verlag, New York.

Slominski, A., Paus, R., Plonka, P., Handjiski, B., Maurer, M., Chakraborty, A., and Mihm, M. C., Jr. (1996). Pharmacological disruption of hair follicle pigmentation by cyclophosphamide as a model for studying the melanocyte response to and recovery from cytotoxic drug damage in situ. *J. Invest. Dermatol.* **106,** 1203–1211.

Southard-Smith, E. M., Kos, L., and Pavan, W. J. (1998). Sox10 mutation disrupts neural crest development in Dom Hirschsprung mouse model. *Nat. Genet.* **18,** 60–64.

Steel, K. P., Davidson, D. R., and Jackson, I. J. (1992). TRP-2/DT, a new early melanoblast marker, shows that steel growth factor (c-kit ligand) is a survival factor. *Development* **115,** 1111–1119.

Streisinger, G., Singer, F., Walker, C., Knauber, D., and Dower, N. (1986). Segregation analyses and gene-centromere linkage in zebrafish. *Genetics* **112,** 311–319.

Sugimoto, M., Uchida, N., and Hatayama, M. (2000). Apoptosis in skin pigment cells of the medaka, *Oryzias latipes* (Teleostei), during long-term chromatic adaptation: the role of sympathetic innervation. *Cell Tissue Res.* **301,** 205–216.

Tachibana, M. (2000). MITF: A stream flowing for pigment cells. *Pigment Cell Res.* **13,** 230–240.

Tachibana, M., Takeda, K., Nobukuni, Y., Urabe, K., Long, J. E., Meyers, K. A., Aaronson, S. A., and Miki T. (1996). Ectopic expression of MITF, a gene for Waardenburg syndrome type 2, converts fibroblasts to cells with melanocyte characteristics. *Nat. Genet.* **14,** 50–54.

Takada, S., Stark, K. L., Shea, M. J., Vassileva, G., McMahon, J. A., and McMahon, A. P. (1994). Wnt-3a regulates somite and tailbud formation in the mouse embryo. *Genes Dev.* **8,** 174–189.

Takeda, K., Yasumoto, K., Takada, R., Takada, S., Watanabe, K., Udono, T., Saito, H., Takahashi, K., and Shibahara, S. (2000).

© 2001 Elsevier Science. All rights reserved.

Induction of melanocyte-specific microphthalmia-associated transcription factor by Wnt-3a. *J. Biol. Chem*. **275,** 14013–14016.

Tan, J. C., Nocka, K., Ray, P., Traktman, P., and Besmer, P. (1990). The dominant W^{42} *spotting* phenotype results from a missense mutation in the c-*kit* receptor kinase. *Science* **247,** 209–212.

Tassabehji, M., Newton, V. E., and Read, A. P. (1994). Waardenburg syndrome type 2 caused by mutations in the human microphthalmia (MITF) gene. *Nat. Genet*. **8,** 251–255.

Tassabehji, M., Read, A. P., Newton, V. E., Harris, R., Balling, R., Gruss, P., and Strachan, T. (1992). Waardenburg's syndrome patients have mutations in the human homologue of the Pax-3 paired box gene. *Nature* **355,** 635–636.

Taylor, M. L., and Metcalfe, D. D. (2000). Kit signal transduction. *Hematol. Oncol. Clin. North Am*. **14,** 517–535.

Thomas, K. R., and Capecchi, M. R. (1990). Targeted disruption of the murine int-1 proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development. *Nature* **346,** 847–850.

Tsujimura, T., Hirota, S., Nomura, S., Niwa, Y., Yamazaki, M., Tono, T., Morii, E., Kim, H. M., Kondo, K., Nishimune, Y., *et al.* (1991). Characterization of *Ws* mutant allele of rats: A 12-base deletion in tyrosine kinase domain of c-*kit* gene. *Blood* **78,** 1942–1946.

Tsujimura, T., Morii, E., Nozaki, M., Hashimoto, K., Moriyama, Y., Takebayashi, K., Kondo, T., Kanakura, Y., and Kitamura, Y. (1996). Involvement of transcription factor encoded by the *mi* locus in the expression of c-kit receptor tyrosine kinase in cultured mast cells of mice. *Blood* **88,** 1225–1233.

Udono, T., Yasumoto, K., Takeda, K., Amae, S., Watanabe, K., Saito, H., Fuse, N., Tachibana, M., Takahashi, K., Tamai, M., and Shibahara, S. (2000). Structural organization of the human microphthalmia-associated transcription factor gene containing four alternative promoters. *Biochim. Biophys. Acta* **1491,** 205–219.

Vaglia, J. L., and Hall, B. K. (1999). Regulation of neural crest cell populations: Occurrence, distribution and underlying mechanisms. *Int. J. Dev. Biol.* **43,** 95–110.

Vaglia, J. L., and Hall, B. K. (2000). Patterns of migration and regulation of trunk neural crest cells in zebrafish (*Danio rerio*). *Int. J. Dev. Biol.* **44,** 867–881.

Verastegui, C., Bille, K., Ortonne, J. P., and Ballotti, R. (2000). Regulation of the microphthalmia-associated transcription factor gene by the Waardenburg syndrome type 4 gene, SOX10. *J. Biol. Chem*. **275,** 30757–30760.

Vrieling, H., Duhl, D. M., Millar, S. E., Miller, K. A., and Barsh, G. S. (1994). Differences in dorsal and ventral pigmentation result from regional expression of the mouse agouti gene. *Proc. Natl. Acad. Sci. USA* **91,** 5667–5671.

Watanabe, A., Takeda, K., Ploplis, B., and Tachibana, M. (1998). Epistatic relationship between Waardenburg syndrome genes MITF and PAX3. *Nat. Genet.* **18,** 283–286.

Wehrle-Haller, B., Meller, M., and Weston, J. A. (2001). Analysis of melanocyte precursors in *Nf1* mutants reveals that MGF/KIT signaling promotes directed cell migration independent of its function in cell survival. *Dev. Biol.* **232**, 471–483.

Wehrle-Haller, B., and Weston, J. A. (1995). Soluble and cell-bound forms of steel factor activity play distinct roles in melanocyte precursor dispersal and survival on the lateral neural crest migration pathway. *Development* **121,** 731–742.

Weston, J. A. (1970). The migration and differentiation of neural crest cells. *Adv. Morphog.* **8,** 41–114.

Wright, S. (1917). Color inheritance in mammals. *J. Hered.* **8,** 224–235.

Wu, M., Hemesath, T. J., Takemoto, C. M., Horstmann, M. A., Wells, A. G., Price, E. R., Fisher, D. Z., and Fisher, D. E. (2000). c-Kit triggers dual phosphorylations, which couple activation and degradation of the essential melanocyte factor Mi. *Genes Dev*. **14,** 301–312.

Yanagisawa, H., Yanagisawa, M., Kapur, R. P., Richardson, J. A., Williams, S. C., Clouthier, D. E., de Wit, D., Emoto, N., and Hammer R. E. (1998). Dual genetic pathways of endothelin-mediated intercellular signaling revealed by targeted disruption of endothelin converting enzyme-1 gene. *Development* **125,** 825–836.

Yasumoto, K., Amae, S., Udono, T., Fuse, N., Takeda, K., and Shibahara, S. (1998). A big gene linked to small eyes encodes multiple Mitf isoforms: Many promoters make light work. *Pigment Cell Res.* **11,** 329–336.

Yasumoto, K., Mahalingam, H., Suzuki, H., Yoshizawa, M., and Yokoyama, K. (1995). Transcriptional activation of the melanocyte-specific genes by the human homolog of the mouse Microphthalmia protein. *J. Biochem*. **118,** 874–881.

Yasumoto, K., Yokoyama, K., Shibata, K., Tomita, Y., and Shibahara, S. (1994). Microphthalmia-associated transcription factor as a regulator for melanocyte-specific transcription of the human tyrosinase gene. *Mol. Cell. Biol.* **14,** 8058–8070.

Yasumoto, K., Yokoyama, K., Takahashi, K., Tomita, Y., and Shibahara, S. (1997). Functional analysis of microphthalmia-associated transcription factor in pigment cell-specific transcription of the human tyrosinase family genes. *J. Biol. Chem*. **272,** 503–509.

Yoshida, H., Kunisada, T., Kusakabe, M., Nishikawa, S., and Nishikawa, S. I. (1996). Distinct stages of melanocyte differentiation revealed by analysis of nonuniform pigmentation patterns. *Development* **122,** 1207–1214.

Received for publication May 8, 2001
Revised August 3, 2001
Accepted August 3, 2001
Published online September 26, 2001

© 2001 Elsevier Science. All rights reserved.