Salamander pigment patterns: how can they be used to study developmental mechanisms and their evolutionary transformation?

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ABSTRACT Neural crest cells of ectothermic vertebrates give rise to three types of pigment cells: melanophores, xanthophores, and iridophores. In early larval salamanders, these cells can combine to generate a variety of pigment patterns across taxa, including vertical bars and horizontal stripes. Such patterns offer an opportunity to study neural crest morphogenesis and differentiation, as well as the evolution of these processes and the morphologies that arise from them. This review examines the phylogenetic distribution of specific pigment patterns, our current understanding of the mechanisms underlying these patterns, and how evolutionary transformations of these mechanisms may have resulted in alternative pigment patterns across taxa.

KEY WORDS: *evolution, lateral line, melanophore, neural crest, xanthophore*

Introduction

Comparative studies of development are essential for evaluating the generality of developmental mechanisms. For example, one particularly exciting discovery in recent years has been the presence of *Hox* genes in a wide variety of metazoans, and the apparently conserved role these genes play in early pattern formation (e.g. Kenyon, 1994). Comparative studies have also revealed a fascinating diversity in the mechanisms of gastrulation (Wray and Raff, 1991), mesoderm formation (Purcell and Keller, 1993), and neural crest morphogenesis (see below) that in some cases was unappreciated even a few years ago. In turn, an understanding of how developmental mechanisms vary across taxa can provide insights into how these mechanisms and the morphologies that arise from them have changed evolutionarily.

In this review, I present salamander pigment patterns as an emerging system for studies at the interface of developmental and evolutionary biology. I begin by examining some of the features of salamander pigment patterns that make them an especially interesting and tractable system for studying basic developmental processes and their evolutionary transformation. Then, I describe some of the events and processes that may contribute to the appearance of pigment cells and the patterns they form. Finally, I review the phylogenetic distribution of specific pigment patterns, our understanding of the developmental mechanisms underlying these patterns, and how differences in these mechanisms may translate into taxon-specific phenotypes. Throughout, I emphasize recent findings from other model systems that may ultimately shed light on the development and evolution of pigment patterns in salamanders.

Salamander pigment patterns as a model system

Pigment patterns of ectothermic vertebrates result from the numbers, spatial arrangements, and ultrastructural characteristics of three types of pigment cells, or chromatophores. These are black melanophores, yellow xanthophores, and silvery iridophores, and in salamanders these cells can generate a striking array of patterns across taxa, both during the larval period and after metamorphosis. Because pigment patterns that occur shortly after hatching have received the most attention, this review will focus only on these early larval patterns (e.g. Fig. 1). Nevertheless, a deeper appreciation for the evolution of pigment patterns will ultimately require an understanding of how post-metamorphic patterns are generated (e.g. DuShane, 1943; Lehman and Youngs, 1959), and whether patterns at different stages of the life cycle are coupled developmentally (e.g. Cowley and Atchley, 1992; Ebenman, 1992).

Abbreviations used in this paper: NC, neural crest; ECM, extracellular matrix; FN, fibronectin; CS-PG, chondroitin sulfate proteoglycan; HA, hyaluronan; NGF, nerve growth factor; SLF, Steel Factor; TGFß, transforming growth factor-ß.

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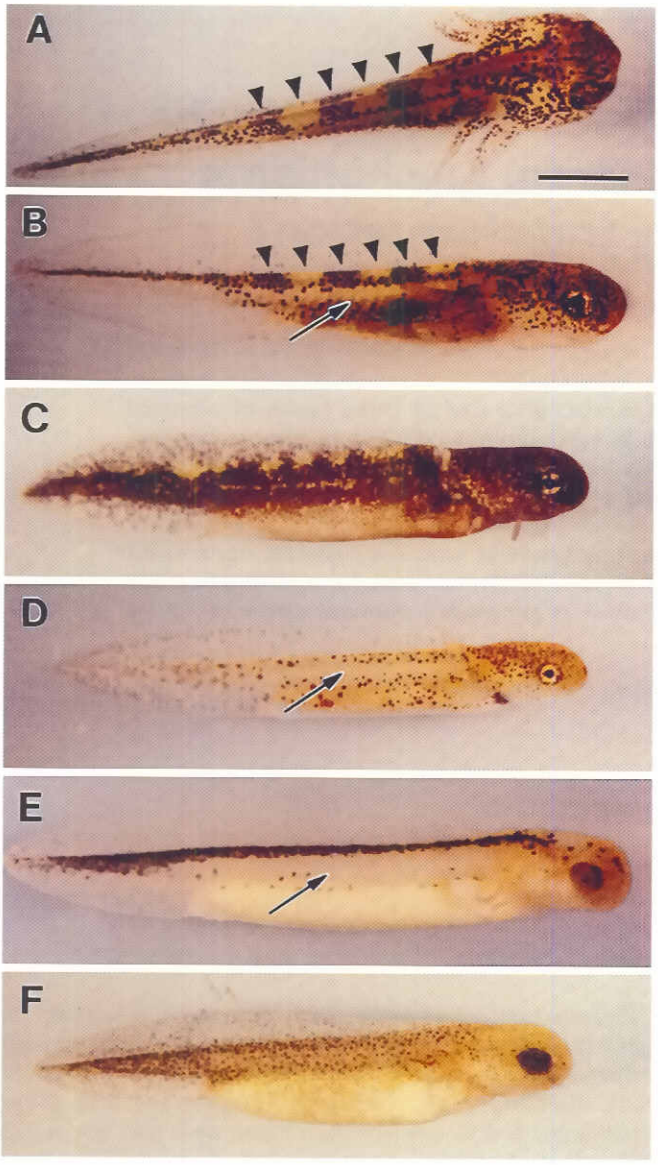

Fig. 1. Diverse pigment patterns of early larval salamanders. (A) *Ambystoma t. tigrinum, dorsal view.* **(B)** *A. t. tigrinum, side view.* **(C)** *A. maculatum.* **(D)** *A. barbouri.* **(E)** *Taricha torosa.* **(F)** *T. rivularis.* **(A-C)** *Alternating vertical bars of melanophores and xanthophores are found in several ambystomatid salamanders (indicated with arrowheads in* **A** *and* **B**). **(B,D,E)** *Horizontal stripe patterns and midbody regions that are relatively free of melanophores (arrows) are found in both ambystomatids and salamandrids. Scale bar, 2 mm.*

In addition to their diversity, salamander pigment patterns are also an attractive system because these patterns arise principally from cells that migrate, proliferate, and differentiate immediately beneath the integument. Many dynamic features of pigment pattern formation can thus be seen even as the phenotype develops in the living organism. Moreover, because salamander embryos are exceptionally large, and develop both slowly and oviparously, they are particularly well suited for experimental manipulations.

Finally, pigment patterns are interesting from both evolutionary and developmental perspectives because pigment cells of salamanders and other vertebrates are derived from neural crest (NC) cells (reviewed in DuShane, 1943; Hall and Hörstadius, 1988; Erickson, 1993; Selleck *et al.*, 1993). As detailed below, NC cells arise along the dorsal neural tube shortly after neurulation, then disperse along stereotypical pathways to various regions within the embryo. In addition to pigment cells, NC cells give rise to an exceptionally diverse array of other cell types and contribute to a wide variety of tissues and organs, including neural and non-neural components of the peripheral nervous system, as well as the craniofacial skeleton, teeth, heart, endocrine glands, and the dorsal fin of amphibians.

During or even prior to their migration, NC cells encounter a variety of factors in their extracellular environment that can influence the precise routes they take, as well as their state of differentiation. The epigenetic nature of NC development suggests several intriguing questions concerning how pigment patterns evolve. For example, *do changes in pigment patterns result principally from changes that are intrinsic to the NC, or through alterations in the extracellular environment the cells encounter? Are evolutionary modifications to pattern-forming mechanisms manifested in other characters that are either derived, or not derived, from the NC? Have superficially similar patterns arisen through common (parallel) or different (convergent) changes in developmental mechanisms? Finally, do the basic features of NC morphogenesis or differentiation make some transformations of development and morphology more likely than others?* By addressing these questions we can achieve a fuller understanding of how pigment patterns evolve and, more generally, how patterns of morphological variation and covariation arise both within taxa and across phylogenetic lineages (e.g. Wake, 1991; Cowley and Atchley, 1992). However, insights into these problems require an understanding of the developmental mechanisms that give rise to specific morphologies, and how these mechanisms vary across taxa. With respect to pigment patterns, this means identification of the factors that govern the morphogenesis and differentiation of NC cells and their pigment cell derivatives.

Morphogenesis of salamander neural crest cells

Origins

Formation of early larval pigment patterns can be viewed as a continuous process that begins with the appearance of NC cells. In all vertebrates, NC cells are derived from the ectoderm and at some stage of development undergo an epithelial-to-mesenchymal transformation by which they segregate from surrounding epithelia. In salamanders, prospective NC cells arise at the border between neural and epidermal ectoderm, and their segregation appears to begin at very early stages since rounded, presumptive NC cells can be identified even within the rising neural folds (Hirano and Shirai, 1984; Spieth and Keller, 1984; Moury and Jacobson, 1990). This differs from amniotes and teleosts in which individual trunk NC cells segregate only after the neural tube has formed (reviewed in Erickson, 1986; Eisen and Weston, 1993). Since NC cells in the trunk are principally responsible for pigment patterns on the flank (DuShane, 1943), the present review will focus only on this subpopulation and will not examine cranial NC development.

As neurulation proceeds and the neural folds fuse at the midline, NC cells form a wedge within the dorsal neural tube. The cells then organize into a distinctive but transient cord, or "crest," running anteroposteriorly just above the neural tube, and it is from this premigratory position that the cells disperse to various destinations within the embryo. As discussed below, differences in the organization and behavior of cells within the premigratory cord can have profound consequences for pigment patterns that arise during later development. Interestingly, NC cells of amniotes fail to assemble into a premigratory cord and instead migrate relatively promptly from their site of origin (Erickson, 1986; Hou and Takeuchi, 1994).

Migratory pathways

For pigment patterns to form, NC cells must disperse from their premigratory position above the neural tube. Unfortunately, migrating NC cells cannot be distinguished unambiguously from other mesenchymal cell types until they begin to differentiate. A variety of methods have thus been used to identify NC cells while their migration is underway. These include immunohistochemical or *in situ* staining with probes specific for NC cells, chimeras with species- or strain-specific markers, and vital labeling with radioactive tracers or dyes. Taken together, studies using these approaches have shown that in avian, chelonian, mammalian, and teleost embryos, NC cells migrate along two general pathways: (i) a "medial" pathway, between the somites and neural tube or notochord; and (ii) a "dorsolateral" pathway, between the ectoderm and the somites (e.g. Sadaghiani and Vielkind, 1989; Serbedzija *et al.*, 1989, 1990; Eisen and Weston, 1993; Erickson *et al.*, 1992; Hou and Takeuchi, 1994). In salamanders, NC cells follow both of these pathways and at later stages are also found within the dorsal fin (Detwiler, 1937; Löfberg *et al.*, 1980). Nevertheless, probes that specifically recognize salamander NC cells have not been identified, and although chimeras and vital dyes were used by early workers, these methods have not been employed recently to further elucidate the details of NC migration in these organisms. Thus, with the exception of NC cells that have started to differentiate into melanophores or xanthophores (see below), we still understand comparatively little of the timing and precise pathways taken by salamander NC cells, or the fates and potentialities of individual cells at different stages of development. For example, Löfberg *et al.* (1980) observed that in *A. mexicanum*, NC cells are present in the dorsolateral pathway before they are found in the medial pathway. This sequence is the reverse from that seen in avian and teleost embryos, and certainly demands further examination. In a similar vein, NC cells that contribute to pigment patterns of most vertebrates migrate along the dorsolateral pathway (Erickson, 1993). Yet, it seems likely that externally visible iridophores lining the peritoneum of older salamander larvae probably migrated instead along the medial pathway (Tucker and Erickson, 1986a). Although cells traveling medially in the zebrafish, *Danio rerio*, can give rise to all types of trunk NC derivatives, including melanophores and other pigment cells (Raible and Eisen, 1994), this possibility has yet to be investigated in salamanders.

General controls of NC morphogenesis

Most or all of the melanophores and xanthophores that contribute to pigment patterns on the middle and dorsal flank of young larvae arise from NC cells that have either migrated along the dorsolateral pathway, or have remained dorsal to the neural tube (see below). An understanding of the factors governing the morphogenesis of this subpopulation of NC cells is therefore critical to understanding the development and evolution of taxon-specific pigment patterns. Some insights into these controls have been provided by Löfberg *et al.* (1985). These authors replaced dorsal epidermis from *A. mexicanum* embryos at a stage prior to NC dispersal with developmentally more advanced donor epidermis, either from a more anterior region of an embryo at the same stage, or from an older embryo in which NC cells had just started to migrate. When these embryos were examined later — but still prior to normal dispersal — NC cells could be seen projecting out over the somites only in regions covered by the grafted tissue. A similar precocious spreading of cells was also observed if subepidermal extracellular matrix (ECM) from older embryos was adsorbed onto Nuclepore filters *in vivo*, and these "microcarriers" grafted adjacent to the premigratory NC of younger embryos.

The findings of Löfberg *et al.* (1985) are consistent with the hypothesis that during normal development, changes in the subepidermal ECM are necessary for the onset of NC dispersal. The molecular and physical composition of the ECM also could influence the rate and pattern of NC migration and the localization of these cells at their final destinations. Neural crest cells of salamanders (Tucker and Erickson, 1986a,b; Perris and Johansson, 1990) and other taxa adhere to and migrate on a variety of ECM molecules *in vitro*, including fibronectin (FN), vitronectin, and laminin, as well as collagens type I, IV, and VI, and all of these occur in the vicinity of early and later migrating NC cells in amniote embryos (e.g. Delannet *et al.*, 1994; Tosney *et al.*, 1994; reviewed in Erickson and Perris, 1993). In *A. mexicanum*, migrating NC cells interact with interstitial matrix fibrils as well as the basal laminae that develop both around the neural tube and beneath the epidermis (Löfberg *et al.*, 1980, 1985), and FN, laminin, and collagen type I have been detected in the subepidermal ECM prior to and during NC migration (Epperlein *et al.*, 1988a; Perris *et al.*, 1990). Thus, changes in the abundance of these or other "adhesive" ECM components could conceivably regulate NC dispersal and migration.

A variety of "anti-adhesive" components of the ECM could influence NC morphogenesis as well. For example, tenascin, chondroitin sulfate proteoglycans (CS-PGs), and hyaluronan (HA) reduce the adhesion of NC cells to ECM substrata and can modulate the motility of these cells *in vitro* (Tucker and Erickson, 1984; Halfter *et al.*, 1989; Perris and Johansson, 1990). Although the mechanisms underlying such effects are not resolved (see also Yamagata *et al.*, 1989; Chiquet-Ehrismann, 1991; Morris, 1993), these molecules could potentially enhance motility *in vivo* by reducing the strength of adhesive interactions between NC cells and other ECM components that might otherwise trap them in place. Negatively charged macromolecules like CS-PGs and HA also could decrease the density of interstitial matrices through the creation of hydrated spaces. In salamanders, tenascin has not been identified between the epidermis and the somites at early stages (Epperlein *et al.*, 1988a), and NC cells of other taxa can themselves produce tenascin (Tucker and McKay, 1991), suggesting this molecule does not act as an environmental cue that triggers dispersal, though it may influence pigment cell localization at later stages (see below). In contrast, CS-PGs and

HA are present subepidermally (Perris *et al.,* 1990; Parichy, unpublished data), and in *A. mexicanum* the epidermis produces an exceptionally high molecular weight chondroitin sulfate/dermatan sulfate proteoglycan during the stages of NC migration (Stigson and Kjellén, 1991). This is consistent with a function of these molecules in regulating initial dispersal as well as subsequent migration or settling. Despite accumulating circumstantial evidence for the roles of adhesive and anti-adhesive ECM components, however, the functions of specific salamander ECM molecules in NC and pigment cell morphogenesis have yet to be tested *in vivo*.

Besides differences in the ECM, differences in the competence of NC cells to interact with surrounding matrices could also influence their morphogenetic behavior. One way that cells interact with the matrix is by expressing specific cell–ECM adhesion molecules. Probably the most studied of these are the integrins (reviewed in DeSimone, 1994), each of which is composed of an α and a β subunit. Currently, at least 15 α and 8 β subunits are known, and various combinations of subunits result in adhesive specificities for one or more ECM ligands. This combinatorial complexity is further enhanced because individual cells often express more than one kind of integrin. The complement of integrins expressed by salamander NC cells is not known, but NC cells of other taxa can express at least α_1, α_4, α_5, α_6, α_v, β_1, β_3, and β_5 subunits (Duband *et al.,* 1986, 1992; Bronner-Fraser, *et al.,* 1992; Delannet *et al.,* 1994; Stepp *et al.,* 1994; Joos *et al.,* 1995). Since different integrins can have distinct roles in adhesion and migration (Delannet *et al.,* 1994; Beauvais *et al.,* 1995), developmental regulation of integrin expression could conceivably influence the timing and pattern of NC migration, as well as the ultimate localization of these cells and their derivatives.

In addition to interactions between NC cells and the ECM, interactions among NC cells almost certainly influence morphogenetic events in the dorsolateral pathway. At early stages, dispersal could be inhibited if adhesive contacts are maintained or re-established between segregated NC cells in the premigratory cord (see Duband *et al.,* 1988; Akitaya and Bronner-Fraser, 1992; Delannet and Duband, 1992). At later stages, interactions among NC cells could also promote their dispersal over the flank. In particular, high cell densities at the level of the neural tube could drive NC cells laterally and ventrally, where densities are lower. Consistent with this possibility, avian NC cells exhibit contact stimulated migration (Thomas and Yamada, 1992), and both avian and salamander NC cells display contact inhibition of movement *in vitro* (Tucker and Erickson, 1986a; reviewed in Erickson, 1986). Alternatively, NC cells could release diffusible substances that repel their neighbors, causing a net movement away from regions of high cell density (reviewed in Twitty, 1949). Such negative chemotaxis has not been ruled out in salamanders, though attempts to demonstrate this phenomenon using avian NC cells have not been successful (Erickson, 1986).

Lastly, the morphogenetic behavior of NC and pigment cells also appears to be influenced by growth factors and/or trophic factors produced by other tissues in their environment. For example, transforming growth factor-β (TGF-β) can stimulate production of FN and adhesion to FN, as well as the precocious migration of avian presumptive NC cells, probably by regulating integrin expression or function (Delannet and Duband, 1992; Rogers *et al.*, 1992). *In vitro*, differentiated human melanocytes express the nerve growth factor (NGF) receptor p75, and both migrate chemotactically and increase their dendricity in response to NGF. Since epidermal keratinocytes express NGF, it may contribute to regulating melanocyte behavior during normal development (Yaar *et al.*, 1991). A final candidate for regulating NC and pigment cell morphogenesis is Steel Factor (SLF). In mice, SLF transcripts are detectable in the dermatome just before NC cells disperse into the dorsolateral pathway, and melanocyte precursors express the SLF receptor, c-kit (Wehrle-Haller and Weston, 1995; Kunisada *et al.*, 1996). In avian embryos, SLF is expressed by the epidermis and developing feather filaments, and melanocytes express c-kit as they home to these tissues (Lecoin *et al.*, 1995). Moreover, mutations at the murine loci encoding SLF and c-kit produce defects in hematopoeisis, primordial germ cell development, and pigmentation, the latter of which range from localized white spots to the complete absence of melanocytes in the integument. Studies of mutant and experimentally manipulated embryos (Besmer *et al.*, 1993; Wehrle-Haller and Weston, 1995; Yoshida *et al.*, 1996) as well as NC cells *in vitro* (Morrison-Graham and Weston, 1993; Reid *et al.*, 1996) indicate that effects on pigmentation probably result from a failure of melanocyte precursors to migrate from the vicinity of the neural tube, and/or a failure of these precursors to survive and proliferate. Additional evidence for a role in morphogenesis comes from the findings that SLF can act as a chemotactic agent for other cell types, probably associates with the ECM *in vivo* (see Wehrle-Haller and Weston, 1995), and can alter the expression of integrins by human melanocytes, as well as the affinity of these cells for different ECM substrata (Scott *et al.*, 1994). Effects of SLF may also depend on interactions involving neurotrophins (e.g. NGF, NT-3; Langtimm-Sedlak *et al.*, 1996; see also Yaar *et al.*, 1994). We are currently investigating the roles of homologs to SLF and c-kit during salamander development.

Chromatophore differentiation

To form pigment patterns, NC cells must not only migrate from their site of origin, they must also differentiate. Two general approaches have been used to identify NC cells that have started to differentiate into pigment cells, but have not yet become externally visible (Epperlein *et al.,* 1988b). First, pigment cell precursors, or chromatoblasts, can be identified at the ultrastructural level by the presence of characteristic organelles. Second, histological techniques can sometimes be used to detect melanoblasts and xanthoblasts. Melanoblasts that are already expressing tyrosinase (an enzyme in the melanin synthesis pathway) can be revealed by administering dopa, which is subsequently converted to externally visible melanin. Unfortunately, dopa incubation does not reveal cryptic melanoblasts in some taxa [e.g. *Tr. alpestris* (Epperlein *et al.,* 1988b), *A. maculatum* (Olsson, 1993)], and it also can stain a variety of other cell types including leucocytes and xanthophores. In contrast, xanthoblasts that have started to produce pigments as well as fully differentiated xanthophores can be identified by their autofluorescence under light of the appropriate wavelength (Fig. 2). In living embryos, this autofluorescence lasts only 1-2 min, but can be viewed again after ca. 2 h. If embryos are sacrificed, autofluorescence can be enhanced by immersion in alkaline solution, though tissue

degradation under these conditions hampers later histological examination. As detailed below, dopa incubation and autofluorescence have sometimes proven valuable for detecting melanoblasts and xanthoblasts, respectively. Nevertheless, both methods have severe practical limitations, and only recognize chromatoblasts at relatively late stages of commitment (i.e. once the cells are already competent to produce pigment). Additional early markers are thus needed not only for detecting melanoblasts and xanthoblasts, but also iridoblasts and NC cells that have not yet become committed to particular lineages. The recent identification of several new markers for NC cells or melanoblasts in other taxa (e.g. Kitamura *et al.*, 1992; Mayor *et al.*, 1995; Wehrle-Haller and Weston, 1995) suggests that similar probes may soon be available for salamanders as well.

Despite the limitations of existing methods for detecting chromatoblasts in salamanders, it is clear that populations of NC cells begin to differentiate prior to or shortly after dispersal. Specifically, dopa-positive cells believed to be melanoblasts are found within and adjacent to the premigratory NC cord in *A. mexicanum* (Epperlein and Löfberg, 1984) and *A. t. tigrinum* (Olsson and Löfberg, 1992), and melanophores are observed in the dorsolateral pathway apparently shortly after dispersing in *Taricha torosa* (Tucker and Erickson, 1986a). In all of these taxa, xanthoblasts are also detectable at similar levels. This early commitment to pigment cell lineages is similar to the situation in avian and mammalian embryos, in which markers for melanocyte differentiation are first detected in cells directly above the neural tube (Reedy and Erickson, personal communication) or between the dorsal neural tube and adjacent somites (Kitamura *et al.*, 1992; Wehrle-Haller and Weston, 1995). Indeed, the arrest of salamander NC cells in a premigratory cord may be functionally analogous to the arrest of amniote NC cells in a "migration staging area" lateral to the neural tube. In this region, extracellular cues or the migratory arrest itself are hypothesized to restrict an initially pluripotent NC population to pigment cell fates (Weston, 1991; Erickson and Goins, 1995). In salamanders, factors in the vicinity of the neural tube also could specify NC cells as chromatophores, or could confer upon them locomotory capabilities that allow them to enter the dorsolateral pathway.

What causes salamander NC cells to differentiate into chromatophores? In *A. mexicanum*, NC cells will only differentiate into melanophores and xanthophores if cultured in the presence of serum, or alternatively, if the cells are co-cultured with neural tube, epidermis, or ECM isolated from the dorsolateral (but not medial) pathway (Perris and Löfberg, 1986; Perris *et al.*, 1988). This could indicate that during normal development, structural components of the ECM in the vicinity of the premigratory cord, or lining the dorsolateral pathway, provide a permissive or stimulatory environment for the differentiation of melanophores and xanthophores. Conversely, ECM in the medial pathway could lack such properties, or could specifically repress the differentiation of melanophores and xanthophores. Consistent with the general idea that structural components of the ECM can influence differentiation, melanogenesis proceeds more slowly when avian NC cells are cultured on FN, as compared to laminin or glass (Rogers *et al.*, 1990), and chromatophore differentiation is inhibited if salamander NC cells are cultured in the presence of

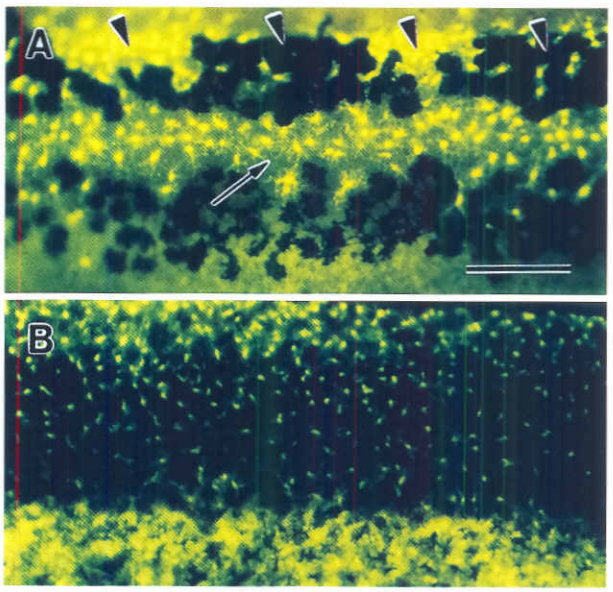

Fig. 2. Xanthophore distributions are revealed by autofluorescence. (A) *Xanthophores in vertical bars and in the midbody "melanophore-free region" in the definitive early larval pattern of A. t. tigrinum.* **(B)** *Xanthophores interspersed with melanophores on the flank of T. rivularis. Symbols as in Figure 1. Scale bar, 0.5 mm.*

HA (Tucker and Erickson, 1986b; see also Adams and Watt, 1993).

In addition to structural components of the matrix, growth factors sequestered within the ECM or otherwise presented along migratory pathways could promote or repress differentiation, and also could contribute to the maintenance or proliferation of NC cells with different potentialities (reviewed in Hall and Ekanayake, 1991; Weston, 1991; Adams and Watt, 1993). In amniotes for example, FGF-2 (Sherman *et al.*, 1993) promotes melanogenesis in NC-derived cells *in vitro*, associates with heparan sulfate, and is present in diverse tissues during development (Kalcheim and Neufeld, 1990). In contrast, the widely distributed TGFβs can inhibit melanogenesis (Stocker *et al.*, 1991), and a member of the TGFβ-family, *dorsalin-1*, is expressed in the dorsal neural tube and can stimulate neural plate cells to migrate and proliferate *in vitro*. After removal of dorsalin-1 from the medium, these cells can differentiate into melanocytes (Basler *et al.*, 1993). It seems likely that these or other growth factors [*e.g.*, SLF, neurotrophins (Langtimm-Sedlak *et al.*, 1996), endothelins (Lahav *et al.*, 1996)] could influence the differentiation of ectothermic NC cells into chromatophores, and could differentially affect the maintenance or proliferation of different pigment cell precursors. To date, however, exceedingly little is known of the distribution or function of growth factors in relation to NC morphogenesis and differentiation in amphibians (but see: Karne *et al.*, 1993; Launay *et al.*, 1994).

Finally, there is circumstantial evidence for several other factors that also could influence the differentiation of cells contributing to early larval pigment patterns. Specifically, the hormone α-MSH stimulates melanization both *in vitro* and during post-embryonic stages of development, and the epidermis and neural tube are α-MSH immunoreactive during NC migration in *A. mexicanum* (Frost-Mason *et al.*, 1992). *In vitro* studies have also shown that melanization can be inhibited by factor(s) in the ventral integument

of adult anurans (Fukuzawa *et al.,* 1995), and possibly the integument of white mutant *A. mexicanum* (Thibaudeau and Frost-Mason, 1992; see also Millar *et al.*, 1995). Differences in the ability of tissues to promote chromatophore differentiation, as well as intrinsic changes in the differentiative behavior of NC cells, could contribute to the evolution of specific pigment patterns by altering the complements of the different chromatophores (e.g. Twitty, 1945; Parichy, 1996a; compare Fig. 1B,C). Nevertheless, the molecular bases for such alterations remain almost completely unexplored.

Pigment pattern elements

A first step towards understanding the development and evolution of pigment patterns is to identify the relevant units for analysis. One approach is to characterize overall patterns according to the presence, absence, or variability of specific pattern elements (e.g. vertical bars or horizontal stripes; see below). Since different pattern elements share a common source of pigment cells and possibly other determinants as well, they are likely to be coupled developmentally. The extent of such non-independence is an empirical question that can be addressed only through experimental or quantitative genetic approaches. Nevertheless, identifying pattern elements can help to categorize pigment pattern diversity, and can guide investigations into pattern-forming mechanisms and their evolutionary transformation.

Vertical barring patterns

One distinctive pattern element seen in some early larvae is a series of alternating vertical bars of melanophores and xanthophores (Figs. 1A-C, 2A). Within salamanders, vertical barring appears to be a novelty shared by most of the taxa for which there exist descriptions in the family ambystomatidae (Fig. 3). Similar barring patterns are also found on the tails of some anuran larvae (Altig and Johnston, 1989) and in many fishes (Riehl, 1991).

How do vertical bars develop?

Development of the vertical barring pattern can be conceptualized as occurring in two phases: (i) the formation of a "pre-pattern" at the level of the premigratory NC; and (ii) the translation of this pre-pattern into the definitive vertical barring pattern on the flank. In the first phase, dopa-positive cells believed to be melanoblasts are initially found above the neural tube, during the early stages of NC migration. As migration proceeds, some of these cells are still detectable in the premigratory position, but others have become widely scattered in the dorsolateral pathway. Simultaneously, aggregates of cells begin to form at variable intervals along the premigratory NC cord. Initially, dopa-positive cells are found within the aggregates, but as the aggregates become more pronounced, autofluorescing xanthoblasts also can be found within them. At later stages, the aggregates contain xanthoblasts or xanthophores but not melanophores. These aggregates represent the prepattern for vertical bars, and have been identified in *A. mexicanum*, *A. t. tigrinum*, *A. annulatum*, *A. barbouri*, and *A. talpoideum* (Epperlein and Löfberg, 1984; Olsson and Löfberg, 1992; Olsson, 1994).

The second phase of vertical bar formation occurs as xanthophores in the periodically arranged aggregates disperse into the dorsolateral pathway, where melanoblasts have already migrated and have started to differentiate into melanophores. Time-lapse videomicrography of melanophore movements (Epperlein and Löfberg, 1990) as well other descriptive studies (Lehman, 1957; Olsson and Löfberg, 1992; Olsson, 1994) have suggested that as xanthophores disperse, melanophores already on the flank recede short distances from the advancing cells. During this process, relatively little intermixing of the two cell types occurs, and xanthophores initially maintain their periodicity on the flank, as specified by the position of aggregates along the neural tube. Gradually, the pattern of alternating bars of melanophores and xanthophores becomes increasingly distinct, and in some taxa xanthophores also invade a "melanophore-free region" that arises in the middle of the flank (see below; compare Figs. 2A, 4B). The "definitive" vertical barring pattern persists from hatching stages though the stage of independent feeding, but is later obscured as melanophores and xanthophores appear in regions previously occupied only by the other cell type (Parichy, in preparation).

The processes governing the two phases of vertical bar development are not well understood. At the level of the premigratory NC, a causal relationship between aggregate morphogenesis and xanthophore differentiation seems likely, but the nature of such a relationship has yet to be established. For example, cells beginning to differentiate into xanthophores could initiate aggregate morphogenesis, or alternatively, the aggregates could themselves provide a microenvironment favorable for xanthophore differentiation. Whatever the precise sequence and causal relationships, several complementary mechanisms could be involved. One possibility is that aggregates form in regions of enhanced proliferation or delayed dispersal, and cells in these aggregates could even be clonally related (see Mintz, 1967; Lin *et al.,* 1992).

Aggregates could also arise through morphogenetic movements within the premigratory NC cord. Indeed, NC cells within the premigratory cord of *A. mexicanum* align parallel to one another in an anteroposterior orientation before they disperse (Spieth and Keller, 1984), perhaps reflecting shifts in the position of these cells along the anterior-posterior axis. If such movements exist, they could result from changes in the relative strengths of cell-cell and cell-ECM adhesion, brought about either through "extrinsic" modifications in the surrounding ECM, or though "intrinsic" alterations in the expression of cell adhesion molecules. Moreover, if different chromatophore types express different adhesion molecules, this could result in the formation of pure populations of melanophores and xanthophores through a sorting-out of differentially adhesive cell types (Steinberg, 1970). For example, cultured xanthophores of *X. laevis* and the teleost *Oryzias latipes* express N-CAM and N-cadherin, whereas cultured melanophores and iridophores fail to express these cell-cell adhesion molecules (Fukuzawa and Obika, 1995). Consistent with a differential adhesion mechanism in *A. mexicanum*, melanophores are located on the periphery, and xanthophores occur internally or basally in aggregates that form when NC cells are prevented from dispersing, either by a genetically defective tissue environment, or by removal of overlying epidermis. This could indicate that during normal development, dopa-positive cells initially within the aggregates "sort-out" and migrate away, leaving behind pure populations of xanthoblasts (Epperlein and Löfberg,

1990). Nevertheless, it remains unclear whether aggregates in mutant or experimentally manipulated embryos arise via the same morphogenetic mechanisms as aggregates during normal development, and dynamic rearrangements have not been reported for aggregates *in vivo*. The contribution of a sorting-out process to the formation of xanthophore aggregates is thus unresolved. Indeed, it has also been suggested that dopa-positive cells fade or disappear within the aggregates (Epperlein and Löfberg, 1984), implying they either die, remain undifferentiated possibly until later stages, or ultimately differentiate into xanthophores. In support of this last possibility, cells in aggregates that develop *in vitro* can contain organelles typical of both melanophores and xanthophores (Epperlein *et al.*, 1988b). Identification of new early markers for these cell types may help to resolve this issue.

Finally, during the second phase of bar formation several different hypotheses could explain interactions between pigment cells on the flank, including contact inhibition of movement, contact stimulation of migration, and negative chemotaxis. These possibilities and the role of dynamic changes in cell adhesion during aggregate morphogenesis remain to be investigated.

Comparative and evolutionary aspects of vertical barring patterns

Vertical barring patterns present an opportunity to study the developmental mechanisms responsible for the presence of a novel morphological character in some taxa, and its absence or loss from others. An important step in such an analysis is to identify the tissues in which relevant evolutionary changes may have occurred. A number of interspecific grafting experiments (e.g. DuShane, 1943; Lehman, 1957) have established that cells within the neural folds are specified for their ability or inability to form vertical bars. This could indicate that evolutionary changes intrinsic to the NC are responsible for vertical barring patterns. However, since neural folds also contain prospective epidermal (e.g. Hirano and Shirai, 1984) and probably neuroepithelial cells, alterations in these tissues still cannot be ruled out.

At the level of the NC, a host of changes could potentially result in a transformation between patterns with and without barring. For example, if xanthophores initiate aggregate formation, evolutionary alterations in the pattern or timing of xanthoblast differentiation could be responsible for the presence or absence of vertical barring. Alternatively, vertical bars could reflect a change in the general organization of NC cells in the premigratory cord, which is cylindrical and several cells thick in taxa that develop aggregates and vertical bars, but appears to be flatter and less distinctive in taxa that lack these characters (Detwiler, 1937; Epperlein and Löfberg, 1990; Olsson and Löfberg, 1992; Olsson, 1993, 1994). Such a change could result from interspecific differences in proliferation rates or the expression of cell adhesion molecules. In turn, aggregates could form if some cells located initially within a denser, multilayered cord become trapped above the neural tube by changes in the surrounding matrix.

It has also been hypothesized that the relative timing of melanoblast and xanthoblast dispersal is causally related to the presence or absence of vertical barring patterns within *Ambystoma* (Lehman, 1957; Olsson, 1993). In particular, melanophores and xanthophores are thought to disperse simultaneously in taxa that lack bars (e.g. *A. maculatum*), whereas xanthophore dispersal is delayed in taxa that form bars (e.g. *A. mexicanum*). Consistent with this possibility, early outgrowths from cultured neural folds of *A. maculatum* contain greater proportions of xanthophores as compared to outgrowths from *A. mexicanum* (Lehman, 1957). Similarly, xanthophores are already scattered over the flank in *A. maculatum* (Fig. 3 in Olsson, 1993) at a stage when xanthophores are found only in the premigratory position in taxa that form vertical bars. Finally, xanthophores are detectable apparently soon after dispersing, and only shortly after the appearance of melanophores in the more distantly related salamandrid *T. torosa* (see below). But since we have yet to visualize the initial dispersal of melanoblasts and xanthoblasts in ambystomatids without bars (see Raible and Eisen, 1994), we still lack direct evidence for a change in the relative timing of melanophore and xanthophore dispersal within *Ambystoma*. Nor do we know how a delay in xanthophore dispersal might be regulated, or precisely how such a delay might translate into a vertical barring pattern. Clearly, a deeper understanding of the mechanisms underlying vertical bar formation will provide new insights into the evolutionary origins of this character in *Ambystoma*, and perhaps in more distantly related anurans and fishes as well.

Horizontal stripe patterns

Many salamanders at early larval stages exhibit horizontal stripes. For the purpose of analysis, these overall patterns can be divided into at least three elements: (i) a dorsal concentration of melanophores along the dorsal myotomes; (ii) a lateral concentration of melanophores near the dorsal margin of the yolk mass; and (iii) a midbody region over the middle of the myotomes, in which melanophores are either absent entirely or occur only sparingly. Since xanthophores are typically abundant in this midbody "melanophore-free region," the resulting pattern consists of a yellow middle stripe bordered by dark dorsal and lateral stripes (Figs. 1B,D,E, 2A). Moreover, because taxa with nearly uniform patterns exhibit melanophores in all regions of the flank (Figs. 1F, 2B), the presence of a "melanophore-free region" can be used to define the presence of a horizontal stripe pattern. Melanophore-free regions (and hence, horizontal stripes) are found in Ambystomatidae, Salamandridae, and Proteidae (Fig. 3), and these patterns also can be "superimposed" on vertical barring patterns (e.g. *A. t. tigrinum*; Fig. 1B). Similar melanophore-free regions bordered by dorsal and lateral concentrations of melanophores are also found in hatchling *X. laevis* (Tucker, 1986) and zebrafish, *D. rerio* (Eisen and Weston, 1993).

How do horizontal stripes develop?

The development of horizontal stripes has been studied extensively in the salamandrids *T. torosa* and *Tr. alpestris*, and more recently in several ambystomatids as well as other salamandrids. In *T. torosa*, melanophores are first visible in the dorsolateral pathway near the neural tube, and xanthophores are detectable at similar levels several hours thereafter (Tucker and Erickson, 1986a; Parichy, 1996a). During early stages, both cell types become relatively uniformly distributed over the flank, but during later development, melanophores become segregated into dorsal and lateral concentrations, whereas xanthophores become localized in the midbody and in the expanding fin. The dorsal

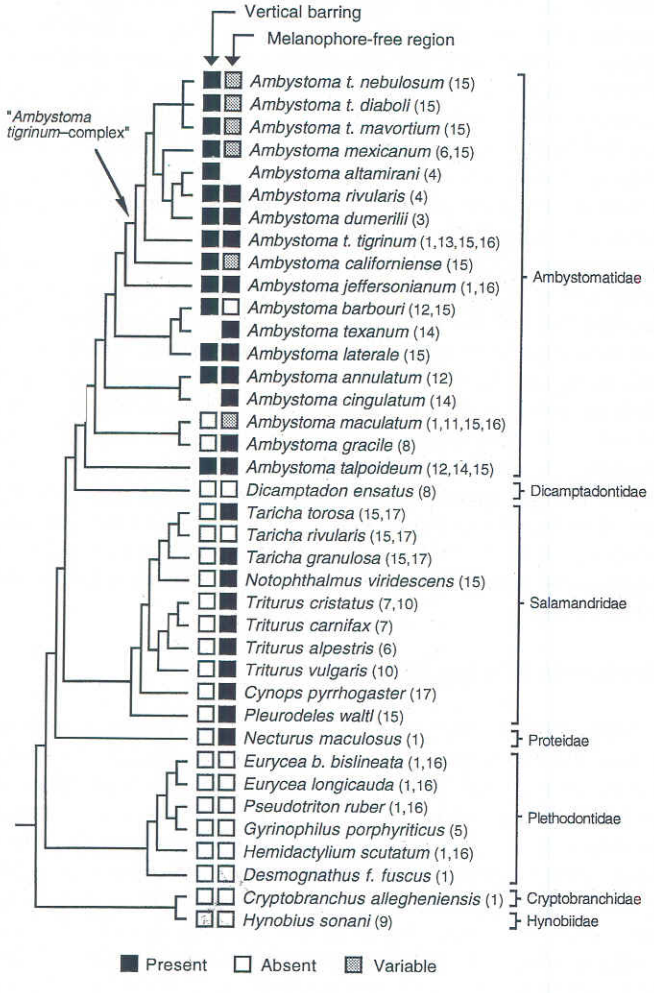

Fig. 3. Phylogenetic distribution of vertical barring patterns and melanophore-free regions. *Character states were assigned based on direct examination of early larvae, or according to published descriptions if accompanied by photographs or illustrations (numbers next to taxa indicate sources; below). The reconstruction of phylogenetic relationships is based on several independent studies (5, 18–27; below) and represents only one of many possible topologies. Included are only those taxa for which data on early larval pigment patterns could be obtained. Salamander families probably diverged between 65–200 Myr BP (Larson, 1991), whereas taxa within the "Ambystoma tigrinum–complex" may have radiated as recently as 0.02–5 Myr BP (27). Vertical bars were scored as either "present" or "absent." Melanophore-free regions (see text) were scored as: "present" if larvae exhibited a distinctive melanophore-free region on the flank; "variable" if intra-taxon variability was found, or if larvae exhibited an indistinct or irregular melanophore-free region; and "absent" if melanophores were densely and nearly uniformly distributed over the flank. A detailed analysis of evolutionary transformations in these and other larval characters will be presented elsewhere. For A. texanum and A. cingulatum, published illustrations of older larvae were judged too ambiguous to assign states for vertical barring. In N. maculosus, melanophore-free regions occur further dorsolaterally on the flank than in other taxa. Dorsal blotches are found in H. scutatum, E. b. bislineata, and D. f. fuscus, but these do not form distinctive stripes or bars. Sources are: (1) Bishop, 1941; (2) Brandon, 1961; (3) Brandon, 1972; (4) Brandon and Altig, 1973; (5) Collazo and Marks, 1994; (6) Epperlein and Löfberg, 1990; (7) Fox, 1955; (8) Henry and Twitty, 1940; (9) Kakegawa et al., 1989; (10) Liozner and Dettlaff, 1991; (11) Olsson, 1993; (12) Olsson, 1994; (13) Olsson and Löfberg, 1992; (14) Orton, 1942; (15) Parichy, 1996a, unpubl. data; (16) Pfingsten and Downs, 1989; (17) Twitty, 1936; (18) Riemer, 1958; (19) Wake and Özeti, 1969; (20) Lombard and Wake, 1986; (21) Macgregor et al., 1990; (22) Larson and Dimmick, 1993; (23) Shaffer, 1993; (24) Reilly and Brandon, 1994; (25) Titus and Larson, 1995; (26) Shaffer and McKnight, 1996; (27) Shaffer, pers. comm.*

concentration of melanophores is composed of cells that initially disperse to this level and remain *in situ*, as well as cells that initially migrate farther ventrally and only secondarily retreat towards the apex of myotomes (Twitty, 1936, 1945). This evacuation of melanophores from the midbody region occurs through the active ventral-to-dorsal translocation of individual cells, although interactions between melanophores may also be involved in a terminal "compaction" to form a well-defined dorsal stripe at later stages (Tucker and Erickson, 1986a; Parichy, 1996a; compare to Twitty, 1945).

Through a series of elegant embryological manipulations, Twitty (1936, 1945) established that at stages prior to NC migration, the dorsal somites and to a lesser extent the neural tube (but not the epidermis) are capable of specifying the site of stripe formation. Once melanophores are uniformly distributed over the flank, however, rotation of the epidermis (with attached melanophores) results in a corresponding rotation of the "dorsal" stripe (Tucker and Erickson, 1986a). Taken together, these findings prompt the hypothesis that dorsal axial or paraxial structures are initially responsible for specifying the position of the dorsal stripe, but this capability shifts to the overlying epidermis during the stages of pigment pattern formation. This could indicate that the somites or neural tube produce a pattern-forming cue that becomes associated with the epidermis, or these structures induce the epidermis itself to produce such a cue. Interestingly, melanophores adhere preferentially to the somites during early stages, but this adhesive specificity gradually shifts to the epidermis as melanophores concentrate dorsally (Tucker and Erickson, 1986a).

The factors controlling the formation of a lateral concentration of melanophores in *T. torosa* were not understood by early workers (Twitty, 1936; Twitty and Bodenstein, 1939). More recently, it has been shown that at least some melanophores are arrested at this level through an interaction with the pronephric duct (Tucker and Erickson, 1986a), which lies just beneath the epidermis during initial stages of pigment pattern formation but is internalized during later stages. Evidence for this interaction comes from the finding that melanophores adhere to the pronephric duct when it is dissected from the embryo, and the cells fail to localize at their normal position along the border of the yolk mass when the duct is ablated.

As in *T. torosa*, melanophores of *Tr. alpestris* undergo a change from a uniform distribution to a horizontal stripe pattern (Rosin, 1943; Epperlein and Claviez, 1982), and somitic mesoderm has similarly been implicated in these cellular rearrangements. In particular, the mesoderm appears to have a repulsive effect on melanophores since placement of a barrier over the somites allows the cells to settle ectopically beneath the

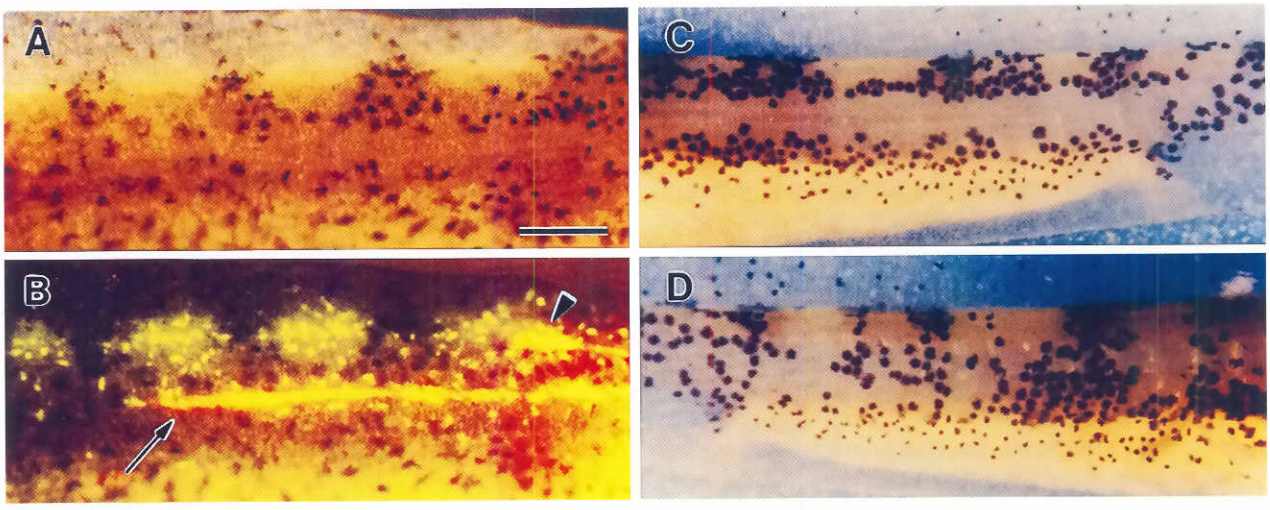

Fig. 4. Interactions between pigment cells and the lateral line system generate the melanophore-free region in *A. t. tigrinum*. **(A,B)** *Formation of the melanophore-free region is temporally and spatially correlated with the migration of the midbody lateral line primordium.* **(A)** *Bright field photomicrograph showing melanophores, vertical bars, and the developing melanophore-free region in the middle of the flank.* **(B)** *Corresponding fluorescence double exposure showing xanthophores as well as the dye-labelled midbody (arrow) and dorsal lateral line primordia (arrowhead).* **(C,D)** *Prevention of lateral line development results in a more uniform distribution of melanophores on the flank.* **(C)** *Unoperated side of an A. t. tigrinum larva.* **(C)** *Opposite side of the same individual on which the lateral line placodes have been ablated, prior to primordium migration and before NC dispersal. Scale bar, 1 mm.*

epidermis in the midbody region (Epperlein and Löfberg, 1990). This suggests that melanophores may normally be inhibited from localizing in the midbody by a pattern-forming cue produced by the myotomes or vasculature, or by a cue produced by the epidermis following an interaction with underlying mesoderm. It would be interesting to replicate this experiment to test for a similar effect in *T. torosa*. This is particularly true since in *Tr. alpestris* the lateral concentration of melanophores does not depend on the pronephric duct (see Epperlein and Löfberg, 1990), melanophores have not been shown to translocate in a ventral-to-dorsal direction during stripe formation (though they do migrate further ventrally; Epperlein and Claviez, 1982), and these salamandrids could differ in other aspects of pattern formation as well.

The factors governing melanophore-free regions (and horizontal stripes) in ambystomatids and other salamandrids have started to be elucidated only recently. Specifically, pigment patterns in these taxa can depend on interactions between pigment cells and the developing lateral lines (Parichy, 1996a,b). In fishes and aquatic amphibians, the trunk lateral lines comprise a bilateral sensory system for detecting mechanical stimuli in the water. These lateral lines arise from migrating lateral line primordia, which originate at ectodermal placodes in the head of the embryo and subsequently travel caudally within the epidermis (Northcutt *et al.*, 1994; Parichy, 1996b; Smith, 1996). As the primordia migrate, they deposit clusters of cells at periodic intervals along the flank, and these clusters ultimately erupt through the epidermis to become mechanosensory neuromasts. In *A. t. tigrinum*, melanophores are widely scattered over the trunk during early stages of pigment pattern formation and prior to migration of the lateral line primordia (Parichy, in preparation). However, vital labeling of the developing lateral lines in conjunction with photographic series of individual embryos shows that the appearance of a melanophore-free region is temporally and spatially correlated with the migration of the midbody lateral line primordium (Fig. 4A,B). Moreover, if lateral line development is prevented, melanophores readily colonize the middle of the flank (Fig. 4C,D). These descriptive studies as well as experimental manipulations indicate the lateral lines influence melanophore distributions initially during primordium migration, and can also act as barriers, preventing melanophores — but not xanthophores — from entering the middle of the flank. Similar though sometimes more subtle effects on melanophores and xanthophores are seen when the lateral lines are ablated in *A. barbouri*, *A. maculatum* and *A. talpoideum*, as well as the salamandrids *Notophthalmus viridescens*, *Pleurodeles waltl*, *T. granulosa* and *T. rivularis*.

What are the molecular mechanisms that govern melanophore behavior during the development of melanophore-free regions and horizontal stripes? One possibility is that melanophores respond chemotactically to growth factors or other substances in their environment. Alternatively, melanophores could follow haptotactic cues in the ECM, or could be trapped by particularly adhesive ECM substrata. For example, higher concentrations of FN reported in dorsal regions of the flank and associated with the pronephric duct could provide especially favorable sites for melanophore localization in *T. torosa* (Tucker and Erickson, 1986a,b). Conversely, melanophore positions also could be specified by anti-adhesive components of the ECM. In particular, deposition of HA and CS-PG in the dorsal fin of *T. torosa* (Tucker and Erickson, 1986b) or tenascin and CS-PG in the midbody of *Tr. alpestris* (Epperlein and Löfberg, 1990) could result in relatively non-adhesive environments, thereby excluding melanophores from these regions. Circumstantial evidence for this possibility is provided by *in vitro* studies in which these matrix components inhibited the migration or adhesion of NC cells or melanophores (e.g. Tucker and Erickson, 1984, 1986b; Epperlein *et al.*, 1988a; Perris and Johansson, 1990). Moreover, CS-PG is associated

with both persistent and transient barriers to NC migration in avian embryos, and embryological manipulations that result in the absence of CS-PG also eliminate barrier function (Oakley *et al.,* 1994). Similarly, enzymatic digestion of chondroitin sulfate *in vivo* allows *Xenopus laevis* melanophores to enter regions where they normally are not found (Tucker, 1986).

If ECM cues govern the migration and localization of pigment cells, at least two complementary hypotheses could explain why xanthophores are found in regions not occupied by melanophores. First, xanthophores could lack the adhesive capabilities of melanophores, resulting in a specific exclusion from substrata to which melanophores adhere more tenaciously. Consistent with this idea, when total numbers of pigment cells are reduced in *Tr. alpestris*, xanthophores readily colonize regions that otherwise would be occupied by melanophores (Epperlein and Löfberg, 1990). A second hypothesis is that xanthophores possess specific migratory and invasive capabilities that allow them to enter and localize in matrices that are inhibitory for melanophores. The notion that migratory capabilities of NC cells can depend on their phenotype is supported by the finding that cultured avian NC cells with different states of specification and differentiated melanocytes can exploit different migratory pathways when back-grafted into the embryo (Erickson and Goins, 1995). Moreover, *T. torosa* xanthophores can migrate significantly farther than melanophores into collagen gels containing CS-PG (Tucker and Erickson, 1986a), and grafting experiments demonstrate that *A. t. tigrinum* xanthophores are more invasive than melanophores *in vivo* (Parichy, 1996b). Such differences in migratory capabilities could reflect differences in the expression of cell–ECM adhesion molecules. Although the complements of adhesion molecules expressed by salamander NC and pigment cells are not known, it is noteworthy that tumor cells transfected with different integrins then grafted into avian embryos can travel along different NC migratory pathways, depending on the types and abundance of integrins they express (Beauvais *et al.,* 1995). Similarly, amniote melanocytes and melanoma cells express a variety of integrins (e.g. Seftor *et al.,* 1992; Hara *et al.,* 1994; Montgomery *et al.,* 1994; Qian *et al.,* 1994), as well as E-cadherin (Tang *et al.,* 1994) and receptors for HA (Thomas *et al.,* 1993), and different adhesion molecules are associated with differences in adhesion, spreading, dendricity, invasiveness, protease expression, and survival. Given these recent findings, it will be especially interesting to examine the role of adhesion molecules in mediating the invasiveness and pattern-forming capabilities of salamander NC and pigment cells.

Comparative and evolutionary aspects of horizontal stripe patterns

Horizontal stripe patterns provide opportunities to examine convergence, parallelism, and the transformation of developmental mechanisms at several phylogenetic levels. For example, the lateral lines contribute to melanophore-free regions in several ambystomatids and salamandrids, but ablation of the lateral lines does not perturb the melanophore-free region in *T. torosa*. This demonstrates that very different interactions at the cellular level contribute to similar pattern elements across taxa. Nevertheless, several lines of evidence suggest the lateral lines have the potential to create a melanophore-free region in *T. torosa*, even though other factors principally mediate pigment pattern formation. Specifically, the lateral lines in this species appear to act as barriers that sometimes prevent scattered ventral melanophores from joining the dorsal stripe (these few cells instead localize in the midbody, near the lateral line). Moreover, if *T. torosa* lateral line placodes are grafted to *A. t. tigrinum* hosts, the developing *T. torosa* lateral lines create a melanophore-free region, as do the host lateral lines. Conversely, when *T. torosa* melanophores are grafted to *A. t. tigrinum* hosts, these donor cells respond to a failure of lateral line development in a manner parallel to *A. t. tigrinum* melanophores; i.e., they more readily colonize the middle of the flank. Taken together, these observations suggest the hypothesis that lateral line effects on chromatophore distributions are a primitive condition in ambystomatids and salamandrids, and there has been an alteration of pattern-forming mechanisms that has resulted in a decoupling of pigment patterns from the lateral lines in *T. torosa*. Such an alteration has probably involved the appearance of novel, extracellular cues for melanophore localization (Parichy, 1996a).

Comparisons at deeper phylogenetic levels also reveal intriguing similarities and differences in the mechanisms leading to melanophore-free regions and horizontal stripes. In zebrafish, there is a distinctive melanophore-free region over the somites and scattered melanophores localize in the vicinity of the midbody lateral line (e.g. Collazo *et al.,* 1994), as in *T. torosa*. Melanophores in the medial migratory pathway can also translocate in a ventral-to-dorsal direction to join the dorsal stripe (Raible and Eisen, 1994), and an influence of somitic mesoderm is suggested by the finding that the *spt* mutation directly perturbs somite development, and yields correlated defects in melanophore distributions (see Eisen and Weston, 1993). In contrast, the melanophore-free region over the myotomes in *X. laevis* develops because, unlike most vertebrates, NC cells in this species do not migrate dorsolaterally between the somites and the ectoderm. Instead, melanophores either remain dorsally at the apex of the myotomes or migrate medially, and only later travel through the somites or around the ventral tip of the myotomes to emerge at the dorsal margin of the yolk mass (e.g. Tucker, 1986; Collazo *et al.,* 1993). Thus, melanophores in *X. laevis* do not undergo a transition from a uniform to a segregated distribution, but the hatchling pattern is instead dictated by a fundamental difference in the pathways of NC migration.

Finally, investigations into the developmental mechanisms underlying horizontal stripes may also shed light on the factors that result in the loss of such stripes and the repeated appearance of nearly uniform pigment patterns (e.g. *D. ensatus*, *T. rivularis*). Grafting experiments between *T. torosa* and *T. rivularis* suggest that NC cells in these taxa are specified for their ability or inability to form horizontal stripes, consistent with the hypothesis that changes intrinsic to the NC are responsible for the evolutionary acquisition of a uniform pigment pattern. Early studies also suggested that *T. rivularis* melanophores differentiate more slowly and arrest at an earlier state of differentiation, as compared to *T. torosa* melanophores (Twitty, 1936, 1945; Twitty and Bodenstein, 1939). This could indicate that a uniform pattern develops because the melanophores of *T. rivularis* never become competent to recognize pattern-forming cues in their extracellular environment, perhaps because they fail to express particular adhesion molecules at the cell surface. It will be interesting to see whether parallel changes in developmental mechanisms have

occurred across taxa with uniform patterns, and whether such changes are causally related to the states of other characters shared by these salamanders (e.g. reduced dorsal fin and absent or vestigial balancers).

Future Prospects

Salamander pigment patterns have tremendous potential as a model system for studying basic developmental mechanisms. They can also provide valuable insights into the evolutionary transformation of these mechanisms and the morphologies that arise from them. However, these latter advances require a deeper understanding of the molecular and cellular bases for NC and pigment cell morphogenesis and differentiation. One approach to this problem is to investigate basic developmental mechanisms in taxa that are readily available (e.g. *A. mexicanum*), and to use this information for generating hypotheses that can be tested in other taxa that are more difficult to work with or to obtain. Finally, it seems likely that findings from other model systems, and particularly mutants in the mouse and zebrafish (e.g. Johnson *et al.,* 1995), will increasingly provide clues to the development and evolutionary diversification of salamander pigment patterns.

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