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REVIEWS

A PEER REVIEWED FORUM

Somite Development in Zebrafish

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ABSTRACT **A full understanding of somite development requires knowledge of the molecular genetic pathways for cell determination as well as the cellular behaviors that underlie segmentation, somite epithelialization, and somite patterning. The zebrafish has long been recognized as an ideal organism for cellular and histological studies of somite patterning. In recent years, genetics has proven to be a very powerful complementary approach to these embryological studies, as genetic screens for zebrafish mutants defective in somitogenesis have identified over 50 genes that are necessary for normal somite development. Zebrafish is thus an ideal system in which to analyze the role of specific gene products in regulating the cell behaviors that underlie somite development. We review what is currently known about zebrafish somite development and compare it where appropriate to somite development in chick and mouse. We discuss the processes of segmentation and somite epithelialization, and then review the patterning of cell types within the somite. We show directly, for the first time, that muscle cell and sclerotome migrations occur at the same time. We end with a look at the many questions about somitogenesis that are still unanswered.**
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Key words: somite; segmentation; somitogenesis; dermamyotome; myotome; sclerotome; muscle; muscle pioneer; fiber type; zebrafish

INTRODUCTION

Although there is underlying segmentation in the trunk of all vertebrates, the segmented nature of fish remains among the most obvious in the adult animal. Even a cursory examination of the filets in a seafood store reveals that the fish trunk is composed of reiterated blocks of muscle, cartilage, and bone. These serially homologous mesodermal segments are derived from somites that form during embryogenesis. Somite formation and patterning have been studied in teleosts and elasmobranchs for over a century because of interest in the evolution of somite development as well as interest in the development of fish muscle in the agricultural industry. Somite development has been studied in elasmobranchs such as sharks, in fish of economic importance such as herring, trout, carp, mullet, plaice, sole, turbot, eel, tambaqui, anchovy, sea bass, and sea bream, and in small freshwater fish from the aquarium trade such as goldfish, rosy barb, and zebrafish that have experimental advantages over the larger teleosts (for reviews, see Bone, 1978; Stoiber et al., 1999). Recently, the zebrafish has emerged as a model for vertebrate development, and the pace of discoveries on the molecular and genetic basis of somitogenesis in zebrafish has accelerated (Holley and Nusslein-Volhard, 2000). Important work in other fish species is ongoing; in addition to intrinsic interest in understanding fish other than zebrafish, this work is extremely valuable for testing the generality of discoveries in zebrafish and for understanding the developmental mechanisms that generate the diversity of forms found among different species (Stoiber et al., 1999). Much of what is known about myotome patterning in zebrafish has recently been confirmed in the European Pearlfish (Stoiber et al., 1998).

A small tropical fish such as the zebrafish has many advantages for studying somitogenesis. Two approaches to understanding somite patterning in zebrafish have proven to be especially fruitful. One approach, which might be called neo-classical embryology, takes advantage of their rapid external development and their transparency. These advantages allow a cell's behavior to be observed by time-lapse microscopy (Roosen-Runge, 1937; Wood and Thorogood, 1994), its fate to be determined by microinjection of vital dyes (Kimmel and Warga, 1987), its position to be manipulated by cell transplantation (Ho and Kimmel, 1993), and its gene expression to be altered by microinjection of mRNA and DNA (Stuart et al., 1990). The other approach, genetics, takes advantage of the ease of maintaining large numbers of animals, the relatively short genera-

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tion time, and the large clutches of embryos that a single pair of fish produce. These advantages, and work by many labs, have led to the development of techniques for mutagenesis, for the identification of mutants (Chakrabarti et al., 1983; Mullins et al., 1994; Solnica-Krezel et al., 1994; Riley and Grunwald, 1995; Fritz et al., 1996; Gaiano et al., 1996), and for the creation of genetic clones (Streisinger et al., 1981), haploid embryos (Streisinger et al., 1981; Corley-Smith et al., 1996), and a genetic map (Postlethwait et al., 1994). More recently, a high-density genetic map containing many types of molecular markers has been developed (Shimoda et al., 1999). This map, along with genomic libraries with large inserts (Amemiya et al., 1999), facilitates relatively rapid positional cloning of novel genes (Talbot and Schier, 1999).

OVERVIEW

The overall process of somite development in zebrafish is similar to that in amphibians, birds, and mammals (see Kimmel et al., 1995). In zebrafish, gastrulation is first visible when the shield is established on the dorsal side of the embryo (Fig. 1). Shield cells are functionally equivalent to the organizer of amphibians and Hensen's node cells in chick (Oppenheimer, 1936; Shih and Fraser, 1996). These cells give rise to the notochord and prechordal plate, and they exert profound patterning influences on surrounding tissues (Mullins, 1999). The paraxial mesoderm develops from cells around the margin of the early gastrula, which converge toward the dorsal side, forming paraxial mesoderm adjacent to the axial mesoderm that is derived from the shield. This convergence of cells toward the future notochord contributes to the anteroposterior extension of the embryo (Kimmel et al., 1990). During this convergent extension the notochord precursors begin to express signaling molecules such as Sonic hedgehog, which exert patterning influences on the paraxial mesoderm. The first somite forms shortly after the end of gastrulation. As somitogenesis continues, the trunk begins to lift off of the yolk and the tail extends. At the end of the first day of development, somite formation is complete and somite patterning nearly so (Fig. 1).

Genetic approaches have identified several genes that are required in zebrafish and other species for the establishment of paraxial mesoderm, for the convergence and extension movements within the paraxial mesoderm, and for the global anterior-posterior patterning of paraxial mesoderm into trunk and tail. These processes have been reviewed elsewhere (Schier and Talbot, 1998; Holder and Xu, 1999; Kodjabachian et al., 1999; Mullins, 1999; Solnica-Krezel, 1999) and will not be discussed here. We start by discussing the process of segmentation and the formation of somites, then discuss the patterning of cell types within the somite, and conclude with a discussion of major questions for future research.

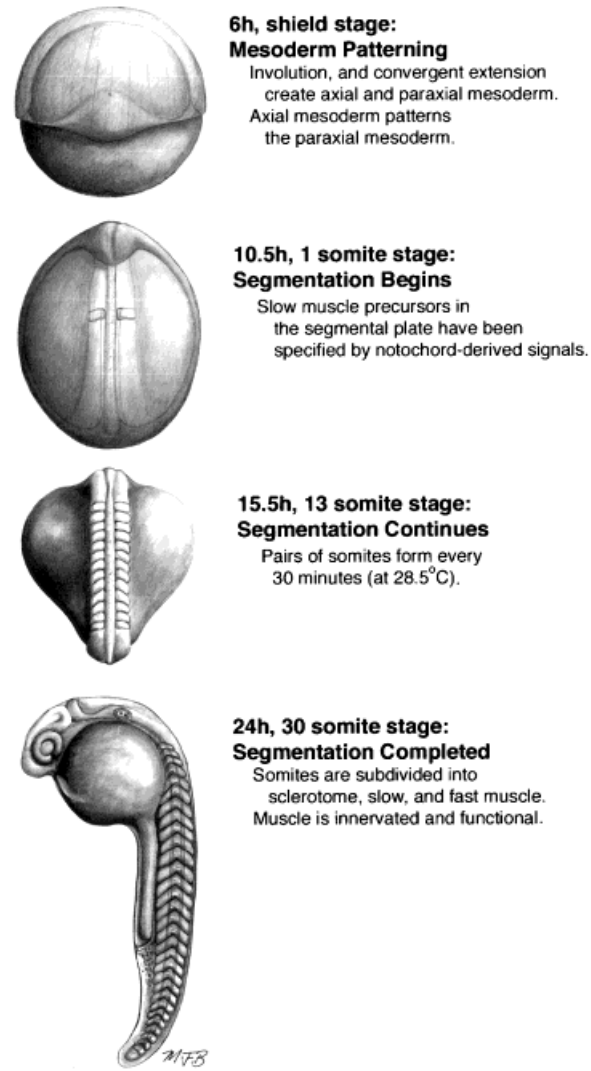

Fig. 1. Overview of zebrafish embryogenesis. These drawings show the stages of embryogenesis during which segmentation and patterning of the paraxial mesoderm takes place. Significant events in each stage are indicated.

SOMITE FORMATION

The first somites in a zebrafish embryo appear approximately 10.5 hr after fertilization. Cells in the extreme rostral region of the presomitic mesoderm (psm) alter their adhesive properties and undergo mesenchymal to epithelial transitions, forming epithelia around loosely organized mesenchymal cells (Fig. 2). Additional somites are produced in a similar fashion at 30 min intervals in a bilaterally symmetric, anterior to posterior wave until a total of about 30 somite pairs bracket the notochord (24 hr; Fig. 1).

A Molecular Clock?

The highly controlled, reiterative nature of somite formation in zebrafish is also characteristic of somite

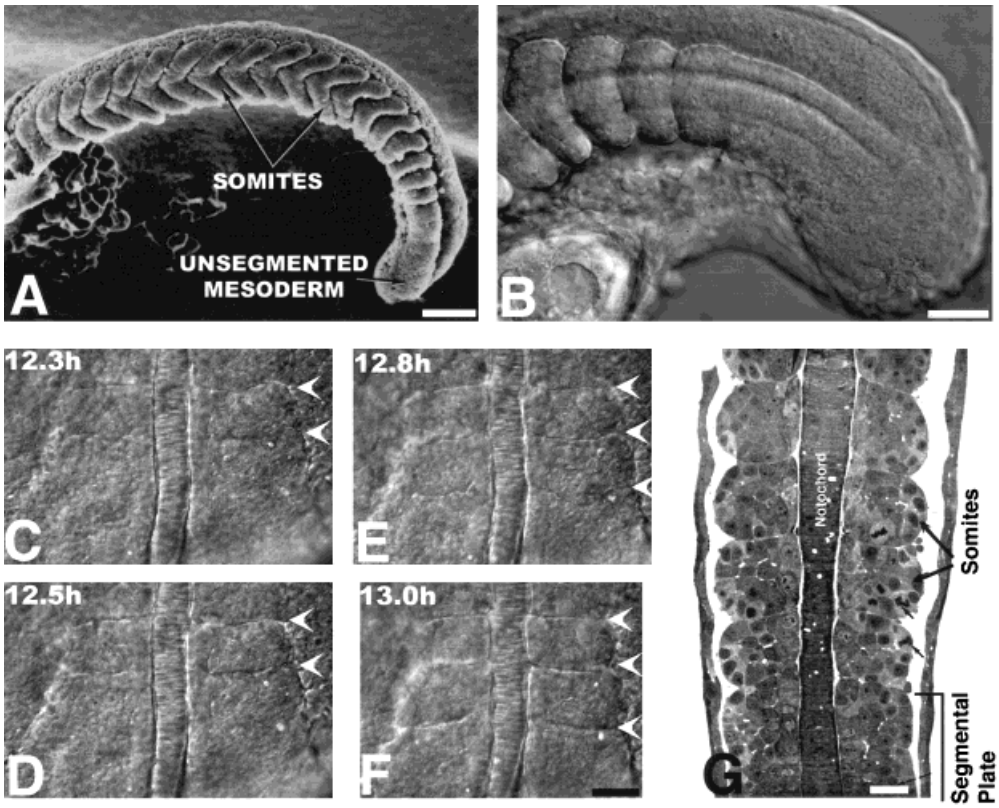

Fig. 2. Overview of segmentation. (**A**) Scanning electron micrograph of a 19 somite embryo. Shortly after somites form, they change from a cuboidal to a chevron shape. Reproduced from Waterman and McCarty (1977) with permission of Scanning Microscopy International. (**B**) Live, lateral view of somitogenesis in a 20 somite embryo. The notochord is out of focus, medial to the somites and presomitic mesoderm. (**C**–**F**) Time lapse views of an embryo undergoing somitogenesis, as observed from dorsal. The notochord, in the center, is flanked on either side by paraxial mesoderm. Arrowheads indicate the positions of somitic furrows. Somitic furrows are first visible in the lateral part of the paraxial mesoderm. (C) Six somites have formed; arrowheads bracket somite 6. (D) The furrow on the right side between somite 6 and the future somite 7 has begun to form in the lateral presomitic mesoderm. (E) The furrow between somite 6 and the future somite 7 is nearly complete on both sides and a new furrow between somites 7 and 8 has begun to form. (F) Somite 7 has fully separated from the presomitic mesoderm. (**G**) Horizontal section through a 20 somite embryo at the level of the notochord. Epithelial boundaries and loosely packed central cells are visible in several of the somites. Reproduced with permission, from Waterman (1969). Scale bars = 100 μm (A); 50 μm (B); 25 μm (C–F); 25 μm (G).

formation in other vertebrate species, and a number of models have been proposed to account for the precise regulation of this process. Several of these models (Cooke and Zeeman, 1976; Meinhardt, 1982, 1986; Keynes and Stern, 1988; Schnell and Maini, 2000) posit the existence of a molecular clock or oscillator that functions with a temporal periodicity. This molecular clock would translate a smooth maturational or positional gradient present in the psm into a spatially periodic pattern, allowing somitogenesis to occur at regular intervals in successive, uniformly sized blocks of cells. The recent descriptions of the expression patterns of *c-hairy1* in chick, *lunatic fringe* in chick and mouse, and *her1* in zebrafish offer support for these models (Palmeirim et al., 1997; Forsberg et al., 1998; McGrew et al., 1998; Aulehla and Johnson, 1999; Holley et al., 2000). First, the expression of each of these genes oscillates in cells in the psm, cycling on and off, with a periodicity equal to the formation time of one somite. Second, at least in the case of *c-hairy1* and *lunatic fringe*, this dynamic expression seems to be an autonomous property of each cell.

Heat shock experiments provide additional evidence for the existence of a clock in zebrafish (Kimmel et al., 1988; Roy et al., 1999). Roy et al. (1999) observed that a single heat shock led to periodic disturbances in somite formation in about 15% of the zebrafish embryos they examined, with boundary defects found in approximately every fifth somite. A somitogenic molecular clock with a temperature sensitive phase can account for this result.

Given these data, a function for some type of clock in vertebrate somitogenesis is now widely accepted. Little is known, however, about the nature of this clock. The oscillation in *her1*, *c-hairy1*, and *lunatic fringe* expression seems to be an output of the clock; these genes do not themselves constitute the clock. In addition, little is known about how the activity of the clock controls somite formation. In most vertebrates, including zebrafish, somite formation seems to occur through the

metameric grouping of cells in the psm followed by the epithelialization of those groups of cells. A number of zebrafish genes, which we discuss below, have been implicated in these events, but the nature of the regulatory link between the activity of these genes and the molecular clock is unclear.

Segmentation of the Paraxial Mesoderm

In zebrafish, like in most other vertebrates, segmentation, the subdivision of the psm into uniformly sized blocks of cells, occurs before the formation of morphologically distinct somites. Metameric patterns of paired bilateral transverse stripes or bands of transcripts have been observed for a number of zebrafish genes, including *deltaC*, *deltaD*, *ephrin-B2*, *EphA4*, *mesp-a*, and *mesp-b* (Fig. 3A; Dornseifer et al., 1997; Durbin et al., 1998, 2000; Haddon et al., 1998; Takke et al., 1999; Sawada et al., 2000), and clearly indicate that cells in the anterior psm are allocated to specific somites before epithelial somite boundaries appear. Recent evidence suggests that the Notch signaling pathway, which has been implicated in mouse and chick somitogenesis, plays a role in the specification of this molecular prepattern in zebrafish.

Notch is a large transmembrane receptor protein that can bind the Delta and Serrate families of transmembrane proteins. Ligand binding causes Notch to be proteolytically cleaved, resulting in the translocation of its cytoplasmic domain to the nucleus. The cleaved Notch protein can then, with the help of a conserved transcription factor (Suppressor of Hairless in *Drosophila*), activate bHLH genes in the *hairy-Enhancer of split* (*E(spl)*) family. In *Drosophila*, the proteins encoded by these bHLH genes complex with a nuclear protein called Groucho to repress transcription of downstream targets (for a review of the Notch signaling pathway, see Artavanis-Tsakonas et al., 1995; see also Struhl and Adachi, 1998).

A number of homologs of components in the Notch pathway have been identified in zebrafish, including four *notch* homologs (Bierkamp and Campos Ortega, 1993; Westin and Lardelli, 1997), four *delta* homologs (Dornseifer et al., 1997; Haddon et al., 1998) and six members of the *hairy-e(spl)* family (the *her* genes; von Weizsäcker, 1994, as cited by Müller et al., 1996). One of the *notch* homologs, *notch1a*, is expressed at high levels throughout the tailbud and psm (Fig. 3A; Bierkamp and Campos Ortega, 1993) and seems to be involved in prepattern specification. Embryos injected with RNA encoding a constitutively active form of Notch1a exhibit no sign of segmentation: no morphologically distinct somite boundaries can be seen, *myoD* expression (normally restricted to the posterior domain of somites) is diffuse in the somitic region, and, in 22–24 hr fish, muscle fibers extend through areas where boundaries should have developed (Takke and Campos-Ortega, 1999). Misexpression of constitutively active *notch1a* also results in the transcriptional activation of *her1* and *her4* throughout the regions in which they are normally expressed in discrete bands (Takke and Campos-Ortega, 1999; Takke et al., 1999).

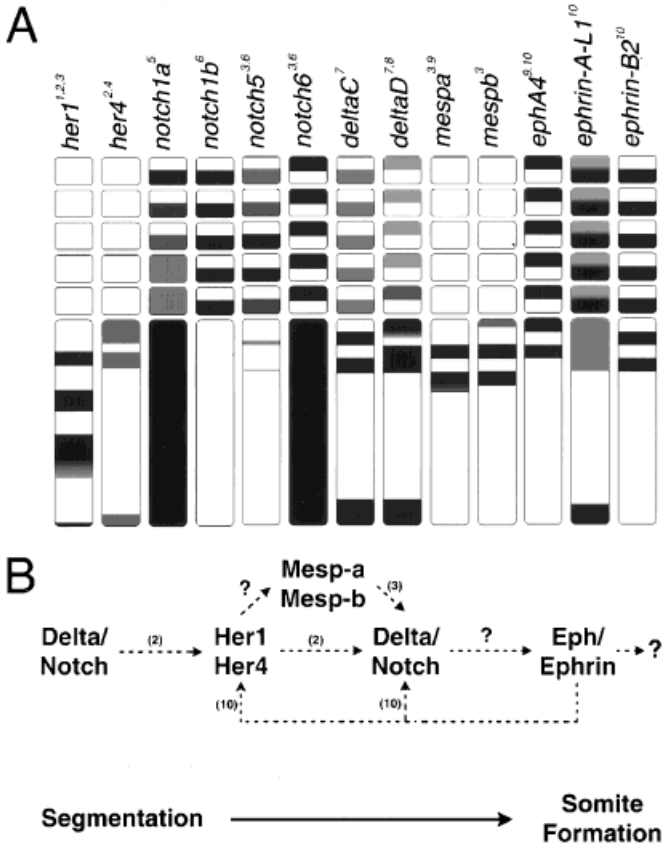

Fig. 3. (**A**) Expression patterns of genes thought to be involved in segmentation and boundary formation. Drawings depict a dorsal view of gene expression in the posterior paraxial mesoderm and are based on published descriptions. Anterior is to the top. The presomitic mesoderm and the five most recently formed somites are shown; adaxial cells are not represented. Shading indicates only gross differences in transcript intensity within the expression pattern of each gene. *her1* oscillates in the psm; the drawing depicts the expression pattern at one moment in time. (**B**) A working hypothesis for a hierarchy of genes involved in somite formation. Dashed arrows are based upon published data (references in parentheses) and are intended to depict only the direction of influence; interactions may be positive or negative and direct or indirect. Dashed arrows with question marks denote speculated interactions (1: Müller et al., 1996; 2: Takke and Campos-Ortega, 1999; 3: Sawada et al., 2000; 4: Takke et al., 1999; 5: Bierkamp and Campos-Ortega, 1993; 6: Westin and Lardelli, 1997; 7: Haddon et al., 1998; 8: Dornseifer et al., 1997; 9: Durbin et al., 2000; 10: Durbin et al., 1998).

Coinjection of *her1* and *her4*, like injection of RNA encoding constitutively active Notch1a, results in the complete absence of somite borders, diffuse *myoD* expression, and perturbation of muscle fiber organization (Takke and Campos-Ortega, 1999). This result, in conjunction with the observed ectopic activation of *her1* and *her4* transcription by overexpression of constitutively active Notch1a, suggests that Her1 and Her4 function downstream of Notch1a in the regulation of psm segmentation (Fig. 3B). Injection of either *her1* or *her4* alone causes less severe disruption of somite de-

velopment (Takke and Campos-Ortega, 1999), indicating that *her1* and *her4* have at least partially distinct roles in the segmentation of the psm.

How is this signaling pathway activated? Two zebrafish homologs of the *Drosophila* Notch ligand Delta, DeltaC and DeltaD, are expressed in the tailbud, in the somites, and in paired bilateral bands in the anterior psm (Fig. 3A; Dornseifer et al., 1997; Haddon et al., 1998). They are obvious candidates for a Notch1a ligand, and loss of *deltaD* function in *after eight* (*aei*) mutants, which have a mutation in their *deltaD* gene, results in the loss of oscillation in *her1* expression (Holley et al., 2000). However, neither overexpression of *deltaC* nor overexpression of *deltaD*, affects the expression of either *her1* or *her4* (Dornseifer et al., 1997; Takke and Campos-Ortega, 1999).

If the regulation of DeltaD activity in the posterior psm is post-transcriptional, then this would explain why the loss of *deltaD* leads to a loss of *her1* oscillation and segmentation whereas the overexpression of *deltaD* has no effect on *her1* oscillation or segmentation (although *deltaD* overexpression does affect the later process of epithelialization, see below). In this scenario, overexpression of *deltaC* and *deltaD* mRNA would not be expected to have any effect on the regulation of *her1* or *her4* transcription as the injected RNA would be subject to normal post-transcriptional regulation. A mutation in the *deltaD* gene, on the other hand, should affect *her1* and *her4* transcription as no normal DeltaD protein could be made. The fact that endogenous *deltaC* and *deltaD* transcripts are not detected in the posterior psm supports a post-transcriptional regulatory mechanism. If transcription of *deltaC* and *deltaD* is not occurring in cells in the posterior psm, any regulation of DeltaC and DeltaD activity there has to be post-transcriptional. The DeltaC and DeltaD proteins required by those cells for Notch activation are likely generated when the cells are located in the tailbud and transcribing *deltaC* and *deltaD* RNA.

A number of questions regarding the involvement of the Notch signaling pathway in the subdivision of the psm have yet to be answered. Do DeltaC and DeltaD in fact act as Notch1a ligands in prepattern specification? What genes lie upstream of the Notch pathway? What genes lie downstream of Her1 and Her4? *her1* does not seem to be expressed in the primordia of the first four segments. How is this tissue segmented? Does the Notch signaling pathway play a role?

Formation of Morphologically Distinct Somites

The second and final step in somite formation is the creation of morphologically distinct somites (i.e., the epithelialization of the presomitic segments). This step seems to involve anteroposterior regionalization within the presumptive and formed somites, as the juxtaposition of two anterior or two posterior somite halves in chick results in extensive cell mixing, whereas a boundary is generated between anterior and posterior somite halves (Stern and Keynes, 1987). In zebrafish, the expression patterns of a number of genes, including *notch5*, *deltaC*, *deltaD*, and *notch6*, clearly demonstrate that anteroposterior subdivisions are present in presumptive and formed somites (Fig. 3A; Dornseifer et al., 1997; Westin and Lardelli, 1997; Haddon et al., 1998). Moreover, early epithelial somite boundaries are not generated in *fss*-type (*fused somites*) mutants, a group of mutants with a seemingly normal segmental prepattern but disrupted anteroposterior segment identity (van Eeden et al., 1996, 1998; Durbin et al., 2000; Sawada et al., 2000). Boundary formation in embryos mutant in the *fused somites* (*fss*) gene, in which the mesoderm of each segment is posteriorized, can be rescued by transplantations of clusters of cells expressing *EphA4*, a gene expressed in the anterior of each segment (Durbin et al., 2000). It is likely that cells in the anterior and posterior regions of each segment differ in adhesive properties and that this difference contributes to boundary formation between the posterior cells of the newly formed somite and the anterior cells of the segmental plate.

Recent evidence indicates that two bHLH transcription factors in the zebrafish Mesp family, Mesp-a and Mesp-b, function in the specification of anterior and posterior identity within each segment. *mesp-b* is expressed in the anterior halves of the three most anterior presumptive somites and seems to confer anterior identity, as expression of the posterior markers *myoD* and *notch5* are reduced in embryos injected with *mesp-b* whereas transcripts of *FGFR1*, *papc*, and *notch6*, all normally expressed in the anterior region of each somite, are uniformly distributed (Sawada et al., 2000). The expression pattern of *mesp-a* is similar to that of *mesp-b*, with two exceptions: *mesp-a* expression does not persist as long as *mesp-b*, so only two bands of expression are observed, and the more posterior *mesp-a* expressing band occupies the entire somite primordium rather than just the anterior half (Fig. 3A; Durbin et al., 2000; Sawada et al., 2000). Durbin et al. (2000) suggest that the pattern of *mesp-a* expression may indicate that anterior and posterior identity within each segment is established approximately 60 min before epithelialization (the time to form two somites).

The expression of a number of zebrafish Notch and Delta homologs is restricted to either the anterior or posterior half of presumptive and formed somites (Fig. 3A; Westin and Lardelli, 1997; Takke and Campos-Ortega, 1999; Sawada et al., 2000), indicating that the Notch signaling pathway may play a role in somite epithelialization in addition to its role in prepattern specification (Fig. 3B). There is some evidence to support a later function. First, misexpression studies indicate that DeltaC and DeltaD may function downstream, as well as upstream, of *her1*. Overexpression of *her1*, *her4*, or RNA encoding a constitutively active form of Notch1a causes defects in the expression patterns of *deltaC* and *deltaD* in the anterior psm (Takke and Campos-Ortega, 1999). Also, somite boundaries

are disrupted in embryos injected with *deltaC* or *deltaD* although, as discussed previously, *her1* expression is not affected (Takke and Campos-Ortega, 1999). Second, overexpression of *mesp-b* causes the downregulation of *notch5* expression and the upregulation of *notch6* (Sawada et al., 2000), suggesting that those two genes have an activity downstream of *mesp-b* activity (Fig. 3B).

The function of the Notch signaling pathway in the translation of the segmental prepattern into epithelial somites is not yet clear. Loss of function studies involving mouse homologs of Delta and Fringe (a *Drosophila* protein known to be involved in Notch signaling in the wing margin) suggest that the Notch signaling pathway is involved in the anteroposterior regionalization of segments (Hrabe de Angelis et al., 1997; Evrard et al., 1998; Kusumi et al., 1998; Zhang and Gridley, 1998; Barrantes et al., 1999). In zebrafish, Notch signaling could act to maintain the regionalization within the somites through a mechanism similar to that employed in the establishment of wing margins of *Drosophila* (Takke and Campos-Ortega, 1999; Rawls et al., 2000). Briefly, in *Drosophila* wing margins the Notch ligand Delta is expressed in ventral cells, activating Notch in dorsal cells, whereas Serrate (another Notch ligand) is expressed in dorsal cells, activating Notch in ventral cells. The Notch signal is then interpreted differently in the two compartments. Delta and Serrate each enhance the expression of the other, and a positive feedback loop is formed between the dorsal and ventral cells that presumably acts to strengthen and maintain the boundary between the two populations of cells. Because zebrafish *deltaC*, *notch5*, *notch1a*, and *notch1b* are expressed in the posterior half of presumptive and formed somites and *deltaD* and *notch6* in the anterior, DeltaC and DeltaD could act in a manner analogous to the *Drosophila* Delta and Serrate (Dornseifer et al., 1997; Westin and Lardelli, 1997; Haddon et al., 1998; Sawada et al., 2000). In such a scenario, DeltaC would activate Notch6 whereas DeltaD activated Notch5, Notch1a, or Notch1b.

Alternatively, the Notch signaling pathway could be directly involved in the epithelialization of the somites rather than in the maintenance of anteroposterior identity within the segment. The analysis of the phenotypes resulting from mutations in the *Drosophila* genes *Notch*, *Delta*, and *Enhancer of split* suggests that in *Drosophila* embryonic tissues these genes are involved in the acquisition or maintenance of an epithelial state (Hartenstein et al., 1992), providing precedence for a Notch function in the epithelialization of zebrafish somites.

The Eph receptor tyrosine kinases and their Ephrin ligands have, like the Mesp family members and Notch signaling pathway, been implicated in the formation of epithelial somites. *ephrin-A-L1*, *ephrin-B2*, and the Eph receptor *ephA4* are expressed in the psm and developing somites of zebrafish in a metameric pattern, and interference with their signaling as well as overexpression of full-length *ephrin-B2* disrupts somite development but not prepattern specification: metameric bands of *her1* expression are still observed (Fig. 3A; Durbin et al., 1998). The anteroposterior regionalization of each segment also seems to be unaffected by the disruption of Eph signaling, as *FGF-8* and *myoD* are still restricted to the anterior and posterior of the somites, respectively (Durbin et al., 1998). Given that transcripts of *ephA4* and *ephrin-B2* are normally regionally restricted in the somites and anterior psm (see Fig. 3A), Eph receptors and Ephrins seem to function downstream of anteroposterior regionalization. Furthermore, their activity seems to be necessary for the downregulation of *her1* and *deltaD* expression in the anterior psm (Fig. 3B; Durbin et al., 1998).

Ephrins and Eph receptors are thought to function intercellularly as repulsive factors. Because EphA4 can function as a receptor for Ephrin-B2 and bands of *EphA4* and *ephrin-B2* expression alternate in both the somites and the psm in zebrafish (Fig. 3A; Durbin et al., 1998), it seems highly likely that these two proteins engage in repulsive intercellular signaling. Such repulsive signaling could function directly in the formation of epithelial boundaries between somites, but it is unclear how this signaling would affect the regulation of *her1* and *deltaD* transcription.

Two other genes, *snail1* and *par1*, may also be involved in the formation of morphologically distinct somites. *par1* encodes a zebrafish homolog of the bHLH transcription factor Paraxis, that in mouse is required for the formation of epithelial somites, but not for segmentation or the establishment of cell lineages (Burgess et al., 1996). It is reasonable to think the same might be true in zebrafish, as high levels of *par1* transcripts are detected throughout newly formed somites and in the psm posterior to the last formed somite, approximately in the region where the next two somites will form (Shanmugalingam and Wilson, 1998). *snail1* encodes a zinc finger protein whose *Drosophila* homolog is required for mesoderm formation. In zebrafish, *snail1* expression is detected at low levels throughout the psm but is restricted to the anterior and posterior epithelial borders of newly formed somites (Hammerschmidt and Nusslein Volhard, 1993; Thisse et al., 1993). The shift from diffuse distribution in the psm to localized distribution at somitic boundaries begins before the somitic borders actually form, suggesting a possible function for *snail1* in the epithelialization of cells at the somite boundaries (Thisse et al., 1993).

Mutants in Somite Formation

A genetic approach to understanding segmentation and somite formation powerfully complements molecular approaches. Mutations in the genes mentioned above can allow specific hypotheses about the function of known genes to be tested. For example, the identification of the *aei* mutation as *deltaD* demonstrated that DeltaD is required upstream of *her1* (Holley et al.,

2000). In addition, a genetic approach can identify novel, or previously unsuspected, genes that are required for somite development. A number of other zebrafish mutants with defects in somite formation, *fused somites* (*fss*), *beamter* (*bea*), *deadly seven* (*des*), and *mind bomb* (*mib*), have been identified. These mutants, including *aei*, form paraxial mesoderm but do not establish early somite boundaries. The *fss* mutation affects all somites; the *bea* mutation interrupts boundary formation posterior to the third or fourth somite; the *aei*, *des*, and *mib* mutations disrupt boundary formation posterior to somites 7–9 (van Eeden et al., 1996).

Some type of segmental prepattern seems to be established in all of these mutants, as they eventually develop irregular somite boundaries and segmented vertebrae (van Eeden et al., 1996). The regulation of *her1* expression, however, is disturbed in these mutants. *her1* expression in the rostral psm of *fss* mutants decays earlier than normal, reducing the number of bands observed at every stage, whereas the paired bands of *her1* expression are completely lost in *bea*, *aei*, and *des*, mutants (van Eeden et al., 1998). Interestingly, *mib* mutant embryos have approximately normal *her1* expression early on, but by the ten somite stage resemble *aei*, *des*, and *bea* mutants (van Eeden et al., 1998).

Anteroposterior segment polarity is also disturbed in all of the *fss*-type mutants. The anterior half of presumptive segments in *fss* mutants is posteriorized, and cells in the anterior psm of *bea*, *des*, and *aei* mutants concurrently express markers of anterior and posterior identity. Regional anteroposterior identity seems to be lost in *mib* mutants as well (Durbin et al., 2000).

SOMITE PATTERNING

Somites give rise to the axial skeleton and the skeletal muscle of the trunk. Zebrafish, supported by the buoyancy of water and their swim bladder, have no use for the robust skeleton needed to support the bigger, drier vertebrates. Instead, they require large muscles to locomote through their relatively viscous aquatic environment. The fish somite is thus predominantly myotome, with sclerotome a relatively minor component. As a result, although the sclerotome lies ventral to the myotome in the somites of both fish and amniotes, the position of each relative to other trunk tissues differs. For example, in amniotes the cells adjacent to the notochord at the time of somite formation form sclerotome, whereas in zebrafish these cells form myotome. Despite these differences in anatomy, similar cellular interactions are implicated in fish and amniote somite patterning.

In vertebrates, the sclerotome is first recognizable in the ventral portion of the somite (Lewis and Bremer, 1927), and gives rise to the vertebrae and the ribs (Dockter, 2000). Zebrafish sclerotome can be identified morphologically shortly after somite formation as a cluster of cells on the ventromedial surface of the somite. *pax9* and *twist* are expressed in this cluster of mesenchymal cells (Nornes et al., 1996; Morin-Kensicki and Eisen, 1997), many of which will migrate dorsally to encircle the spinal cord and notochord, forming the vertebrae (Fig. 4C, F).

In the adult, zebrafish muscle fibers can be subdivided into two broad classes (for a review of fish muscle fiber types, see Bone, 1978). Slow muscle fibers, which are specialized for slow swimming, are found in a wedge-shaped triangle on the lateral surface of the adult myotome (Fig. 8). Fast muscle fibers, used during bursts of rapid swimming, are located in the deep portion of the myotome. Slow fibers are smaller, darker, and more heavily vascularized than fast fibers.

Precursors to adult slow and fast muscle fibers can be identified very early in development. At the end of the segmentation period (24 hr), fast muscle fibers are found in the deep portion of the myotome, whereas slow muscle fibers form a superficial monolayer on the surface of the myotome (Fig. 4D,E). The embryonic slow muscle population can be subdivided into pioneer slow muscle and non-pioneer slow muscle fibers (Fig. 4D). Pioneer slow muscle fibers were first characterized by Waterman (1969) and by the group of van Raamsdonk et al. (1978, 1982) as early developing muscle fibers that differentiate adjacent to the notochord at the level of the future horizontal myoseptum. Much later, these cells were found to express Engrailed proteins (Hatta et al., 1991), and were named muscle pioneers (Felsenfeld et al., 1991) by analogy with cells of the same name in grasshopper, which play a role in organizing both the musculature and its innervation. Available evidence indicates that zebrafish muscle pioneers are required for neither axon guidance (Melançon et al., 1997), nor the proper development of other muscle fibers (Blagden et al., 1997), although they may play a role in the development of the horizontal myoseptum (Halpern et al., 1993). We use the terms "muscle pioneer" and "pioneer slow muscle" interchangeably. As their name indicates, these are a subset of the embryonic slow muscle fibers, and are likely to develop into slow muscle fibers of the adult (Devoto et al., 1996). The three types of embryonic muscle fibers (non-pioneer slow muscle, pioneer slow muscle, and fast muscle) can be unambiguously identified in the zebrafish somite by position, by morphology, and by the expression of specific molecular markers.

Although sclerotomal and myotomal cells are the only cells that have been characterized in the zebrafish somite thus far, other cell types are likely to exist. For example, zebrafish somite cells contribute to blood vessels (Morin-Kensicki and Eisen, 1997), as do chick somite cells (Brand-Saberi and Christ, 2000). The chick somite also contains the dermatome, which gives rise to the dermis of the skin. The dermatome develops from the most superficial cells of the dermamyotome in chick. Nothing is known about the zebrafish dermatome, it is not even clear that a dermatome exists. Waterman (1969) identified a few cells on the surface of the myotome in a 24 hr fish that he called external

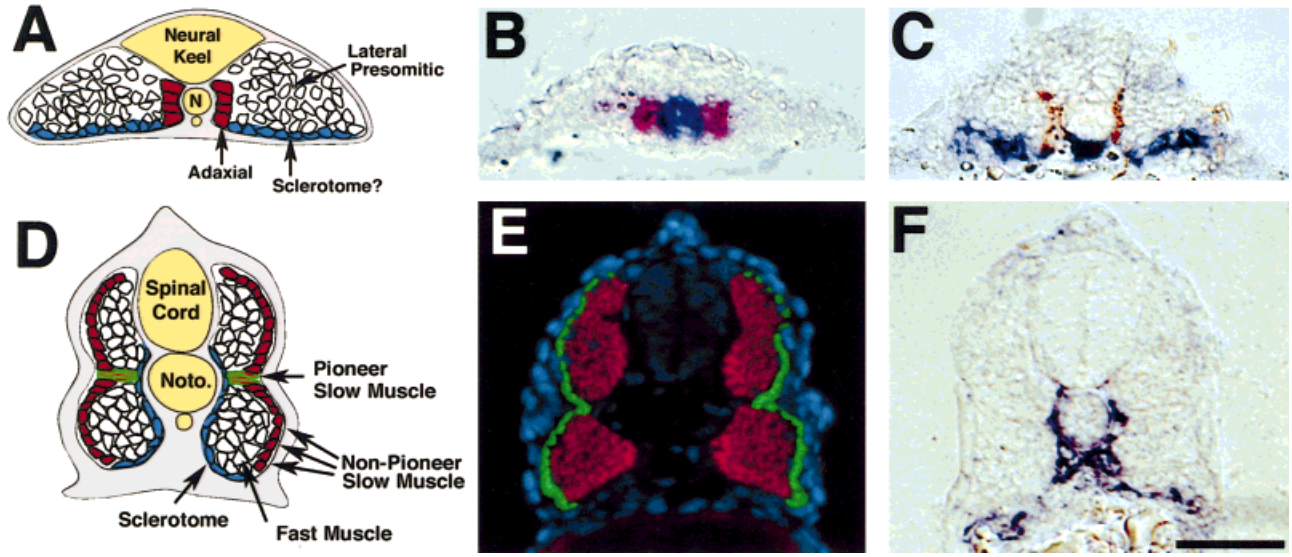

Fig. 4. The zebrafish somite has four characterized cell types. Depicted in this figure are the starting and final positions for cells expressing markers of sclerotome (*twist*), slow muscle (S58, F59) and fast muscle (ZM4). (**A**–**C**) Cross-sections of zebrafish embryos at 13 hr. (A) Schematic cross-section through the anterior psm, showing the relative positions of fast muscle precursors (lateral presomitic), *twist* expressing cells (sclerotome?), slow muscle precursors (adaxial). (B) Adaxial cells express *myoD* (red) while still adjacent to the *shh*-expressing notochord (blue) in the segmental plate. (C) *twist* expressing cells (blue) are initially ventral to the myotome and are separated from the notochord by the medial-most adaxial cells that give rise to slow muscle fibers (brown, F59). Dark blue staining directly beneath the notochord is *twist* labeling of the hypochord. (D–F) Cross-sections of zebrafish embryos at 24 hr. (**D**) Schematic cross-section through a 24 hr zebrafish embryo, showing the positions of the four characterized cell types. (**E**) Slow muscle cells (green) form a superficial monolayer whereas fast muscle cells (red) remain deep. (**F**) *twist* expressing cells (blue) at 24 hr are found ventral and medial to the myotome, such that expression is directly adjacent to the notochord and ventral spinal chord. Dorsal is to the top. Scale bar = 100 μm (B,C,E,F).

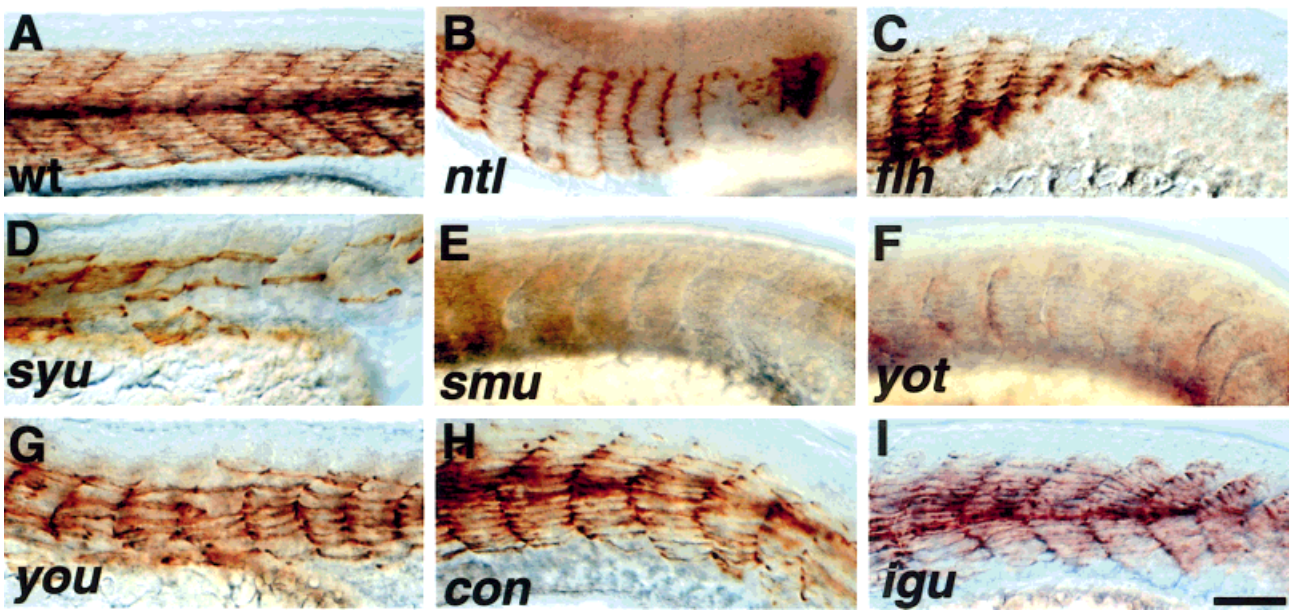

Fig. 5. Slow muscle development is disrupted in several zebrafish mutants with defects in either notochord signaling or the Hh pathway. This figure presents immunolabeling for slow muscle using the S58 antibody in a number of mutants at 24 hr (see also Lewis et al., 1999). (**A**) Wild-type embryo. (**B**,**C**) Notochord mutants. *no tail* (*ntl*) (B) and *floating head* (*flh*) (C) mutants have a loss of axial mesoderm but show early Hedgehog expression and have reduced numbers of slow muscle fibers in the posterior trunk as compared with the more anterior somites. (**D**–**F**) Hedgehog signaling mutants. Mutants in *sonic-you* (*syu*) (D), that encodes the zebrafish homologue of Shh, show partial defects in slow muscle throughout the trunk. *slow-muscle-omitted* (*smu*) (E) and *you-too* (*yot*) (F) mutant embryos possess the greatest deficiency in slow muscle fibers as compared with all other known you-type mutants. *yot* encodes the zebrafish homologue of Gli2, a downstream transcription factor in the Hedgehog pathway, *smu* is proposed to encode zebrafish Smoothened, a transmembrane protein in the Hedgehog receptor complex. (**G**–**I**) Putative mutants in Hedgehog signaling. *you* (G), *chameleon* (*con*) (H), *iguana* (*igu*) (I) all exhibit lesser deficiencies in the number of slow muscle fibers. Reduction in slow muscle is seen throughout the trunk, though the defect tends to be worse in the posterior. Anterior is to the left and dorsal is to the top. Scale bar = 100 μm (A–I).

cells, but whether these are dermatome cells or some other cell type remains to be determined.

Myotome Development

Patterning of the zebrafish paraxial mesoderm begins with the specification of slow muscle precursors before the onset of somitogenesis. Slow muscle precursors can be identified in the segmental plate immediately adjacent to the notochord (Fig. 2C). These cells, which were named adaxial cells by virtue of their position, demonstrate a myogenic identity very early, as indicated by their expression of *myoD* and *myogenin* (Fig. 4B; Weinberg et al., 1996). A few of the adaxial cells per somite develop into pioneer slow muscle fibers (Devoto et al., 1996). The more lateral paraxial mesoderm does not express detectable levels of *myoD* and *myogenin* transcripts until the time of somite formation. Some of the lateral presomitic cells can contribute to both muscle and sclerotome (E. Melançon and SHD, unpublished observations), suggesting that the earliest lineage restriction in zebrafish is between slow muscle and "not slow muscle," and that the latter population is later subdivided into fast muscle and sclerotome.

Shortly after somite formation, adaxial cells undergo a remarkable morphological change and migration; they begin as a sheet of about 20 cuboidal cells all adjacent to the notochord and end as a monolayer of muscle fibers on the surface of the somite (Devoto et al., 1996). The migration of adaxial cells can be divided into two phases: one in which they move dorsally and ventrally while remaining on the medial surface of the somite, and another in which they migrate radially toward the lateral surface of the somite (see Fig. 6B–E for example). Adaxial cells elongate while still located medially, changing their shape from plump cubes into skinny rods (see Fig. 7A,B for illustration). This elongation-driven shape change might be sufficient to displace the adaxial cells dorsally and ventrally. The second phase of adaxial cell migration is the movement of non-pioneer slow muscle fibers radially through the somite. This early movement of mesodermal cells from a medial position to a more lateral position is unusual, although it shares some features with the lateral migration of *single-minded* (*sim*) expressing muscle precursor cells in Drosophila (Lewis and Crews, 1994; Zhou et al., 1997). Interestingly, the *sim*-expressing cells are also dependent on midline signaling (see below).

At about the time that somites form, fast muscle precursors begin to express abundant levels of *myoD* and *myogenin*. This expression is first detectable as segmental bands in the posterior of each somite (Weinberg et al., 1996). These initially lateral myotomal cells differentiate to form the deep part of the myotome as the adaxial cells migrate laterally past them (Devoto et al., 1996; Blagden et al., 1997). Fast muscle fibers can be distinguished from slow muscle fibers by labeling with several fast-specific antibodies, including zm4 (Fig. 4E; Barresi et al., 2000), 12/101 (Devoto et al., 1996) and BA-D5 (Blagden et al., 1997), and by the lack of labeling with slow-specific antibodies, including S58 (Fig. 4E, Fig. 5; Devoto et al., 1996) and EB165 (Blagden et al., 1997). F59 is another muscle antibody that preferentially labels slow muscle but also labels fast muscle faintly in zebrafish (Figs. 4C, 6B–E; Devoto et al., 1996).

Patterning of the Myotome

Cell fate in the somite depends on the cellular environment in which the cell is located. The amniote dermamyotome is patterned by signals from the notochord, the surface ectoderm, the dorsal neural tube, and the lateral plate (Borycki and Emerson, 2000). In zebrafish, the position of slow muscle precursors adjacent to the notochord suggests that notochord signaling might induce slow muscle fate. Support for this hypothesis comes from the characterization of three zebrafish mutants, *floating head* (*flh*), *no tail* (*ntl*), and *bozozok* (*boz*), that exhibit defects in notochord development. In addition to the loss of notochord, mutant embryos have variable deficiencies in early adaxial *myoD* expression, muscle pioneers, and horizontal myosepta (Halpern et al., 1993; Talbot et al., 1995; Odenthal et al., 1996; Blagden et al., 1997). Furthermore, muscle pioneers are rescued in mutant embryos containing transplanted wild-type notochord cells (Halpern et al., 1993).

The notochord patterns surrounding tissues in vertebrates through the secretion of Sonic hedgehog (Shh), a vertebrate homologue of the *Drosophila* segment polarity protein Hedgehog (Hh). In the zebrafish somite, all evidence suggests that Hh proteins induce slow muscle fates. First, the notochord expresses both *shh* and *echidna hedgehog* (*ehh*). Second, overexpression of *hh* mRNA in wild-type embryos results in a dramatic expansion of slow muscle at the expense of fast muscle and presumably sclerotome fates, and slow muscle development in notochord mutants can be rescued by overexpression of *shh* mRNA (Hammerschmidt et al., 1996; Blagden et al., 1997; Du et al., 1997). The number of pioneer slow muscle cells increases in *shh* injected embryos as well, though the cells are still generally localized to the middle of the somite. Third, embryos in which the Hh signaling pathway has been disrupted exhibit defects in slow muscle development. Overexpression of constitutively active PKA, which blocks Hh signaling, results in a loss of slow muscle fibers, as does the hyperactivation of endogenous PKA via treatment with forskolin (Du et al., 1997; Barresi et al., 2000). Moreover, overexpression of Patched, which inhibits Hh signaling, also eliminates slow muscle fibers (Lewis et al., 1999). Mutations in genes encoding components of the Hh pathway lead to defects in slow muscle as well. Null mutations in *syu*, the zebrafish homolog of *shh*, result in partial deficiencies in slow muscle development (Fig. 5; Schauerte et al., 1998; Lewis et al., 1999). This partial phenotype may be the result of redundancy, as *ehh* expressed in the notochord and

twhh expressed in the floor plate may compensate for the loss of *shh*. Mutations in both *you-too*, the zebrafish homologue of *gli2* and a downstream component of the Hedgehog pathway, and *slow-muscle-omitted*, which we have proposed encodes the zebrafish homologue of Smoothened (a part of the Hh receptor complex), lead to an almost complete loss of embryonic slow muscle fibers (Fig. 5; Lewis et al., 1999; Barresi et al., 2000).

Several other mutants display phenotypes similar to *syu*. These mutants are termed the *you*-type mutants because their somites have a U-shape instead of the wild-type chevron shape (van Eeden et al., 1996). As illustrated in Figure 5, mutations in many of the *you*-type genes such as *you* (*you*), *chameleon* (*con*), and *iguana* (*igu*) show variable deficiencies in slow muscle development (Fig. 5; Lewis et al., 1999; Barresi et al., 2000). In addition, many of these mutants can be rescued by overexpression of Hh signaling (Schauerte et al., 1998). Together these results suggest that the *you*-type genes may encode components of the Hedgehog pathway (Schauerte et al., 1998).

The pioneer slow muscle fate may be determined by competing influences between one or more Hh proteins expressed in the midline and one or more BMP proteins expressed near the dorsal and ventral portions of the somite. As discussed above, muscle pioneers develop from a subset of adaxial cells, remaining adjacent to the notochord while the remainder of the adaxial cells migrate radially to the surface (Fig. 4D). We have speculated that these cells receive a longer dose of Hhs and that this additional exposure may be involved in the induction of the muscle pioneer fate (Du et al., 1997). However, if the notochord ectopically expresses *dorsalin-1*, which encodes a BMP4 related protein, adaxial cells adjacent to the notochord are inhibited from developing into muscle pioneers (Du et al., 1997). Because BMP4 related genes are expressed in the dorsal and ventral portions of the myotome, where muscle pioneers do not normally develop (Rissi et al., 1995), it seems likely that the activities of *TGF*-β family members oppose the action of Hh on muscle pioneer fate.

Despite the emergence of this elegant story for slow muscle and muscle pioneer cell induction, a number of questions remain unanswered. Although the Hh pathway is clearly involved in slow muscle induction, the link between Hh signal transduction and slow muscle fate is not yet known. Furthermore, *wnt11*, a member of a family of secreted proteins that has been implicated in vertebrate myotome patterning (reviewed by Sumoy et al., 1999), is expressed in adaxial cells throughout their migration to the lateral surface (Makita et al., 1998). *prox1*, a vertebrate homolog of the *Drosophila prospero* gene, is also expressed in adaxial cells as the cells migrate (Glasgow and Tomarev, 1998). The functions *wnt11* and *prox1* perform are unknown. The remarkable migration of the slow muscle cells is also poorly understood. What causes the cells to migrate? What prevents or delays the muscle pioneers from migrating to the surface of the myotome?

In addition, little is known about fast muscle development. It is possible that Hh and TGF-β signaling are involved in specifying fast muscle fate. Meng et al. (1999) have recently identified a novel zebrafish zinc-finger protein, *terra*, that is expressed in the lateral presomitic mesoderm and in the last 2–3 newly formed somites, but not in adaxial cells (Meng et al., 1999). This expression domain suggests *terra* may play a role in fast muscle development, especially because Hh overexpression results in the reduction or absence of both fast muscle and *terra* expression (Meng et al., 1999). *terra* expression is also eliminated in embryos with mutations in *swirl*, the zebrafish homolog of *bmp2*, indicating that BMP2 is necessary for *terra* expression (Meng et al., 1999). It is conceivable that BMP2 acting through Terra induces a fast muscle fate, but much more research needs to be done. It is also possible that fast muscle is a default state, i.e., cells develop into fast muscle after somite formation if they have not earlier received signals directing them toward a slow muscle or sclerotomal fate.

Sclerotome Development

In amniotes, the ventromedial portion of the epithelial somite undergoes a mesenchymal transition and migrates ventromedially away from the dermamyotome. These mesenchymal cells make up the sclerotome population of the somite and eventually migrate around the notochord and neural tube, giving rise to cartilage and then bone. In zebrafish, like in chick, the ventromedial portion of the somite gives rise to sclerotome cells, that migrate dorsally to surround the notochord and neural tube (Morin-Kensicki and Eisen, 1997). Sclerotomal cells in the anterior portion of the somite migrate before those in the posterior (Morin-Kensicki and Eisen, 1997). Interestingly, some cells in the posterior of the ventromedial cell cluster give rise to muscle fibers (Morin-Kensicki and Eisen, 1997), further suggesting that the cell fate decision between fast muscle and sclerotome is a late one in zebrafish.

The migration of sclerotome dorsally and toward the notochord correlates very closely in time with the migration of adaxial cells away from the notochord. In zebrafish, cells of the ventromedial cell cluster as well as migrating sclerotome cells express *twist* (Morin-Kensicki and Eisen, 1997). *twist* encodes a bHLH transcription factor that in mouse is expressed in the sclerotome (Wolf et al., 1991) and represses the myogenic program (Hebrok et al., 1994; Rohwedel et al., 1995; Spicer et al., 1996; Hamamori et al., 1997). As illustrated in Figure 4A,C, *twist* expressing cells are restricted to the ventral most region of the somite, separated from the notochord by slow muscle cells. These two cell types undergo a dynamic migratory pattern simultaneously, such that slow muscle begins to move laterally while the more ventral *twist*-expressing cells move dorsomedially against the ventral notochord (Fig. 6B,C; Devoto et al., 1996; Morin-Kensicki and Eisen, 1997). When muscle pioneer cells are adjacent to the

notochord, *twist* expressing cells are consistently ventral to the muscle pioneers (Fig. 6D). When muscle pioneer cells move away from the notochord, however, *twist* expressing cells can be found at the same level and dorsal to the muscle pioneer cells (Fig. 6E). Whether muscle pioneer cells block or attract sclerotome cells or whether sclerotome cells are pushing muscle pioneers away from the notochord remains to be determined. The timing and patterns of tissue interactions within the somite are depicted in Figure 7, such that the migration of sclerotome, adaxial cells, motor neurons, and neural crest are illustrated.

Patterning of the Sclerotome

Although Hh from the notochord seems to play a key role in sclerotome induction in amniotes (Murtaugh et al., 1999; Dockter, 2000), little is known about the factors that regulate sclerotome development in zebrafish. During early somitic patterning in amniotes, sclerotomal precursors are adjacent to the notochord and neural tube. In zebrafish, the adaxial cells, a myotomal cell type, are adjacent to the notochord whereas the sclerotome is found ventrolateral to adaxial cells (Fig. 4). This positional difference might lead to differences in the exposure of cells to inductive signals emanating from the notochord and neural tube and raises the question of whether the induction of sclerotome is regulated by Hh in zebrafish as it is in chick.

There is some evidence to suggest that Hh signaling plays a role in sclerotome development in zebrafish. If the Hh pathway is moderately attenuated by treatment with pertussis toxin, sclerotome expands and muscle pioneers fail to form (Hammerschmidt and McMahon, 1998). A stronger block of the hedgehog pathway with constitutively active PKA results in a reduction of sclerotome cells, however, as does hyperactivation of the pathway with Hh overexpression (Hammerschmidt and McMahon, 1998). Whether these effects are a direct action of Hh on sclerotome or the result of an indirect action of Hh on other cell types is unknown.

Several other molecules have been identified that potentially regulate sclerotome development in zebrafish. Tenascin-W, Peripheral myelin protein 22, and Mindin2 are expressed in zebrafish sclerotome and may play a role in sclerotome differentiation or migration (Higashijima et al., 1997; Weber et al., 1998; Wulf et al., 1999). Further characterization of these genes and the identification of sclerotome mutants will provide insights into sclerotome patterning in zebrafish and in amniotes.

Somitic Interactions With Other Cells

We have discussed cell-cell interactions between slow muscle, fast muscle, and sclerotome. In addition, there are several other cell types, such as motor neurons, neural crest cells, and lateral line, that interact with somite cells. Motor neuron growth cones and neural crest cells both move along the medial surface of the somite shortly after somite formation, each with a different destination. The three primary motor neurons send their axons along very stereotyped pathways: one grows dorsally within the somite, one grows ventrally, and one arborizes in the middle (Fig. 7; Eisen et al., 1986). Several observations suggest that these axons detect differences between dorsal and ventral populations of cells. First, motor neurons will find their appropriate target region even if placed into a novel location (Gatchalian and Eisen, 1992). Second, the absence of either sclerotome or muscle pioneers does not perturb axon pathfinding, suggesting that axons are not simply detecting cell-type differences (Melançon et al., 1997; Morin-Kensicki and Eisen, 1997). Third, if the somite is rotated along the dorsal-ventral axis, the pattern of axon outgrowth is disrupted (Beattie and Eisen, 1997). Neural crest also migrates on the somite (Fig. 7; Raible et al., 1992). Crest cells migrate on the lateral as well as the medial surface of the somite, contributing to pigment stripes, dorsal root ganglia, and the enteric nervous system (Raible and Eisen, 1994; Kelsh and Eisen, 2000). Pigment patterning is disrupted when muscle pioneer cells are missing, suggesting that as in chick, neural crest cells respond to the somitic environment in which they find themselves. The lateral line primordium migrates along the lateral surface of the somites in an anterior to posterior direction adjacent to the muscle pioneers (Metcalfe et al., 1985). In the absence of muscle pioneers, this migration is disturbed (Metcalfe and Graveline, 1991), suggesting that the primordium of the lateral line uses somitic cues for its migration. Further understanding of the mechanisms by which motor axons, neural crest, and lateral line primordium cells distinguish between different regions of the somite are likely to illuminate new aspects of somite patterning.

PERSPECTIVES AND FURTHER QUESTIONS

Although our understanding of zebrafish somites has vastly increased since they were first characterized (Waterman, 1969), much remains unknown. As a relative newcomer to model organism status, some simple questions have yet to be answered in zebrafish. We have mentioned some of these above. Larger questions about zebrafish somite development are similar to the major questions about chick and mouse somitogenesis, and we address these below.

Questions of Timing

We have discussed zebrafish mesoderm segmentation, somite epithelialization and somite patterning as if they are distinct processes. This is a very useful conceptual distinction in understanding somite development, and some of these processes can be separated experimentally. For example, mutations or experimental treatments that eliminate somite epithelialization do not disrupt medio-lateral or dorso-ventral somite patterning, and mutations or treatments that disrupt the medio-lateral patterning of the paraxial mesoderm

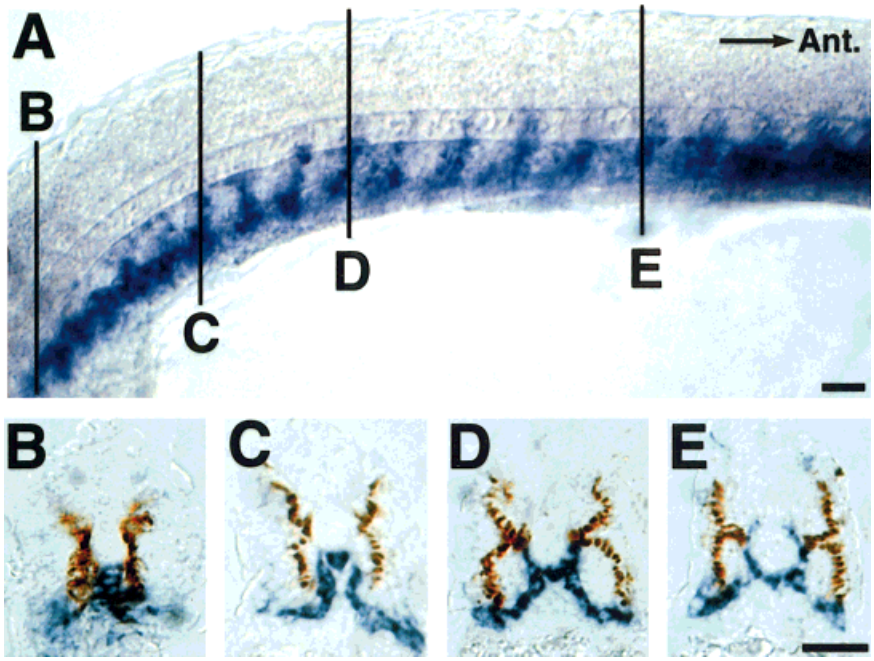

Fig. 6. Correlation between slow muscle migration and sclerotome migration. This figure shows double labeling of slow muscle stained with F59 (brown) and *twist* mRNA (blue) to illustrate the timing of slow muscle radial migration and the apparent movement of *twist* expressing cells dorsally. (**A**) Lateral view of a whole mount in situ on a 21 hr wild-type embryo. Anterior is to the right. The sclerotome in more mature segments has migrated dorsally to the notochord. (**B**–**E**) Representative cross sections corresponding to lines B–E in A. (B) In the tail region, slow muscle cells have begun their dorsal and ventral migration but remain adjacent to the notochord. *twist* expressing cells are positioned ventral to the slow muscle and notochord. (C) As slow muscle begins to move away from the midline, *twist* expressing cells are medial to the ventral-most slow muscle fibers but still ventral to the notochord. (D) In a more anterior section, *twist* expressing cells surround the ventral notochord and abut the muscle pioneer cells, that are still adjacent to the notochord. (E) Only when muscle pioneer cells have begun to separate from the notochord are *twist* expressing cells around the notochord and dorsal to the muscle pioneers. Scale bars = 100 μm (A); 100 μm (B–E).

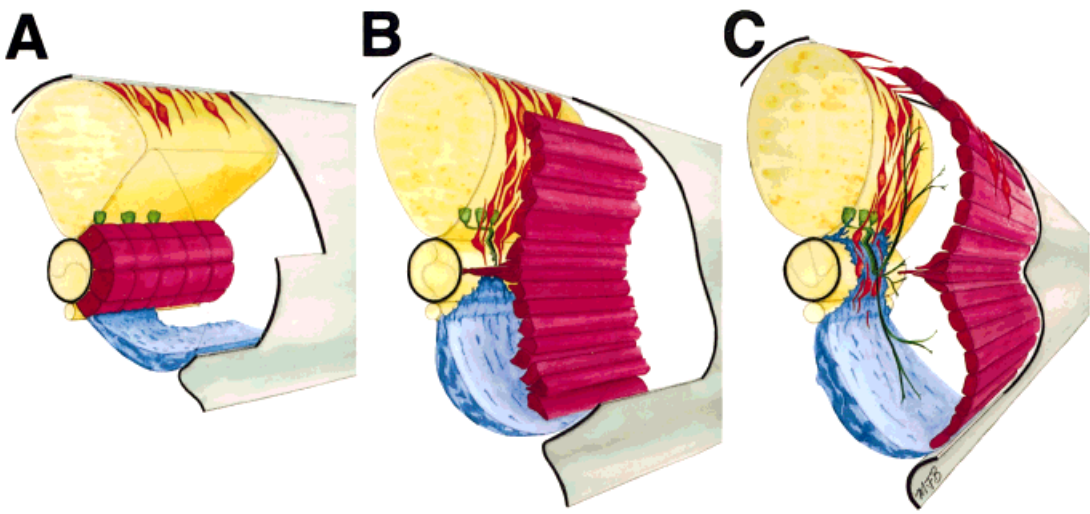

Fig. 7. Multiple populations of cells move at similar times. (**A**–**C**) Schematic views of a developing posterior trunk somite. Neural crest cells are in orange, motor neurons in green, slow muscle cells in red, sclerotome in blue. The clear, 3-dimensional space in the somite represents fast muscle cells. (A) At 12 hr, neither neural crest cells nor motor neuron axons have entered the somite. Adaxial cells are positioned adjacent to the notochord medial to both fast muscle and the sclerotome. (B) At about 18 hr, slow muscle cells are migrating toward the surface with fast muscle precursors positioned both medial and lateral to them. Muscle pioneer cells, however, remain adjacent to the notochord. At this point, neural crest, motor axons, sclerotome converge in the middle of the somite above and below the muscle pioneer cells. (C) Muscle pioneer cells become separated from the notochord at about 24 hr. Neural crest and sclerotome can be seen dorsal and ventral to the middle of the notochord, motor axons extended into the myotome. Neural crest cells can also be seen migrating along their 'lateral pathway' (Raible et al., 1992). Fast muscle is completely medial to the slow muscle fiber monolayer.

do not disrupt segmentation and epithelialization. In the embryo, however, there is a very tight linkage between segmentation and epithelialization, and between epithelialization and somite patterning. The molecular and cellular basis for these linkages is an important area for future research.

We have pointed out some of the suggestive links between the output of the segmentation clock (e.g., *her1*) and gene products that are likely to be involved in events downstream of the segmentation clock (e.g., Notch). Much work remains before the molecular basis of segmentation is fully understood. Moreover, we are far from understanding how the events of segmentation lead to epithelialization. Epithelialization is a result of a nearly simultaneous change in the adhesive properties of large numbers of segmental plate cells. This change in adhesion must involve a change in cell or matrix adhesion molecules, but whether some of the molecules we have discussed above, such as Ephs and Ephrins, play a role is unclear. In addition, the mechanism by which segmentation genes regulate the expression or function of adhesion molecules is unknown. Many of the genes defined by mutations that disrupt somitogenesis in zebrafish are likely to play a role in the process of somite formation. Learning the identity of the genes affected by these mutations is likely to provide valuable insights into the links between segmentation and somite formation.

Cell type specification within the paraxial mesoderm is tightly linked in time to the process of somite formation. In quail, this patterning occurs only after somite formation, when cells become competent to respond to Hedgehog (Borycki et al., 2000). In zebrafish, although slow muscle precursors are induced long before segmentation, specification of other cell types in the paraxial mesoderm occurs shortly after segmentation, as in chicks. For example, slow muscle and sclerotome migration, muscle pioneer differentiation, and fast muscle differentiation all occur within a few hours after somite formation. If the genes that underlie cell differentiation and movement are regulated in part by the transcription factors that are part of the segmentation process, this would ensure a linkage between these different aspects of patterning.

Questions About Later Developmental Events

By about 24 hr of development, the subdivision of the zebrafish somite is essentially complete: both slow and fast muscle fibers are functional and innervated, and sclerotomal cells are migrating to envelop the neural tube. In many important respects, somite patterning is complete. There will be tremendous growth in the myotome and in the sclerotome, however, before the 24 hr zebrafish reaches its adult size between 2 and 6 months later (Fig. 8). The cross-sectional area of the myotome increases by a factor of at least 300 and the length of the trunk by a factor of at least 10, leading to at least a 3,000-fold increase in the volume of somite-derived tissue. This increase is comparable to the increase in size that occurs in birds and mammals during fetal, neonatal, and juvenile growth.

Fig. 8. Somite growth. Cross-section of a 2-month-old zebrafish trunk (approximately segment 12), stained to show muscle (F59). The slow muscle is present as a more darkly stained triangle at the lateral edge of the horizontal myoseptum. The inset shows the same segment at 24 hr at the same scale, demonstrating the tremendous increase in size that occurs during post-embryonic growth. Scale bar = 300 μm.

Muscle growth in all vertebrates occurs by a combination of hyperplasia, an increase in the number of muscle fibers, and hypertrophy, an increase in the size of existing fibers. In fish, as in birds and mammals, hyperplasia plays a larger role in growth during the larval/fetal period, whereas hypertrophy dominates during juvenile and post-juvenile growth. Whereas in amniotes hyperplasia ceases soon after birth, however, in zebrafish hyperplasia continues to play a role during the juvenile period of growth (Rowlerson et al., 1997). The cells that contribute the nuclei necessary for this growth derive from muscle satellite cells, which are undifferentiated myogenic cells that lie between the basal lamina and the membrane of differentiated muscle fibers (Koumans and Akster, 1995). These cells originate largely in the somite in chick, but it is not clear when their precursors are set aside, or what cellular or molecular factors regulate their development

in the early embryo. The cellular resolution of fate mapping that is possible in zebrafish will help determine the embryonic source of satellite cells.

In amniotes, cells of the lateral dermamyotome at the level of limbs differentiate only after they migrate out of the somite and into the developing limb. Zebrafish have two pairs of appendages that are homologous to the two pairs of limbs of tetrapods, the pectoral and pelvic fins. The pectoral fins develop from fin buds that are adjacent to somites 2–4 at 30 hr of development. The musculature of the zebrafish fins develops in the third day (SHD, unpublished observations), but if and how this fin musculature is derived from the somite remains to be seen. The fin is relatively simple in comparison to amniote limbs, and thus may provide a useful system in which to resolve outstanding questions about the development of limb muscle. For example, single-cell labeling techniques in zebrafish will provide a detailed fate map of individual muscle fibers, such that it should be possible to determine when the identity of specific muscle fibers or specific fiber types is specified. Mutants that disrupt the patterning of the non-muscle portion of the limb can be used to test whether the muscle is intrinsically patterned, or derives all of its patterning information from the local environment of the limb.

Comparative Questions

In considering whether an increase in our understanding of zebrafish somite development can further our understanding of amniote somite development, we need to evaluate how fundamental the differences between the zebrafish and the amniote somite are. The most obvious differences are anatomical: in zebrafish the large myotome is adjacent to the notochord, whereas in chick the large sclerotome is adjacent to the notochord. In addition, myogenesis begins before somite formation in fish whereas in chick it likely begins after somite formation. It is not clear whether these differences are a result of deep differences in the mechanisms underlying somite patterning or relatively superficial differences in proliferation and timing. Understanding the basis for the differences is likely to increase our understanding of the developmental mechanisms that underlie evolutionary change.

We choose to emphasize the similarities between fish and amniotes. In both, the dermamyotome is specified first (Devoto et al., 1996; Dockter and Ordahl, 2000), and is dorsal to the sclerotome. In both, the medial portion of the future myotome is the first to differentiate (Ott et al., 1991; Pownall and Emerson, 1992; Weinberg et al., 1996). In both, these early myotomal cells migrate away from the medial surface of the myotome (Devoto et al., 1996; Kahane et al., 1998). In both, *engrailed* is expressed in the dorsal-ventral middle of the myotome (Hatta et al., 1991; Gardner and Barald, 1992). In both, Sonic hedgehog and BMP-like signaling regulate myotomal cell fate (Du et al., 1997; Reshef et al., 1998).

In the past five years it has become increasingly clear that animals with diverse body plans have many underlying conserved developmental mechanisms, including, for example, the involvement of BMPs in dorsoventral patterning in both insects and vertebrates (Holley and Ferguson, 1997). As differences in somite patterning between amniotes and zebrafish are relatively local ones, a reasonable hypothesis is that the mechanisms that pattern the somite in zebrafish will also be involved in patterning the somite in amniotes. For this reason it is fortunate that the advantages and disadvantages of studying somite development in chicks or mice are not the same as those in zebrafish. The ability to do targeted gene knockout in mice provides a very nice "reverse" genetic approach that complements the "forward" genetic approaches of zebrafish. The relatively easy tissue fate mapping and transplantation experiments possible in chick are complemented by the relatively easy cellular fate mapping and transplantation experiments possible in zebrafish. The many tissue culture models of somite cell differentiation in mouse and chick allow very refined molecular, in vitro approaches that complement the cruder, but in vivo approaches that are possible in zebrafish. Thus, questions that are difficult to address in one system may be much easier to address in another. Rapid progress in our understanding of somite development in all vertebrates will be made in the coming years by taking advantage of the opportunities that each system offers.

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