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Somite Development: Constructing the Vertebrate Body

Meeting Review

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Designing a body plan is an architectural challenge. Both invertebrates and vertebrates have addressed this problem by first establishing repeated units of equivalent identity (segments), and later coordinating these motifs into regionally specialized and integrated structures. The most distinct feature of vertebrate mesodermal segmentation is the somite. Indeed, cell fate in the immature somite is flexible and dependent on local environmental signals. Consequently, somitogenesis has generated considerable interest and the somite now serves as a paradigm for investigating how naive cells adopt identity. Somites were first defined at the beginning of the last century, and much of our understanding of somite development comes from morphological observations and experimental manipulations in avian embryos (see Christ and Ordahl, 1995), and more recently, from embryo culture and genetic studies in mice (see Gossler and Hrabé de Angelis, 1997). The rapidly developing zebrafish model promises to unite these approaches.

Important landmarks in somitogenesis are periodicity, segmentation, epithelialization, and differentiation. Somites form pairwise within the presomitic mesoderm (PSM; segmental plate in avians), and on either side of the neuraxis until midgestation (50 in chick, 65 in mouse, and up to 500 in snakes). As a consequence of gastrulation, mesenchymal somite precursor cells feed into the caudal PSM, progressively condense as they move rostrally, and concomitantly somites exit as epithelial spheres from the rostral-most portion of the PSM (Figure 1; see below). Subsequently, cells oriented toward the notochord differentiate into the sclerotome via an epithelial–mesenchymal transition. Underneath the surface ectoderm, the remaining epithelium forms the dermomyotome, which later contributes to skeletal muscle and dermis. Current progress in the understanding of somitogenesis was discussed at a recent meeting on Somite Development organized by O. Pourquié (Developmental Biology Institute of Marseille, France) on the mediterranean island of Les Embiez (France; Oct., 1997).

Somites Are Born after Gastrulation

Gastrulation, which begins with the formation of the primitive streak, leads to mesoderm formation and sets the scene for somitogenesis. Several groups investigated these early stages using microsurgical techniques. P. Tam (Children's Medical Research Institute, Wentworthville, Australia) and G. Schoenwolf (University of Utah) reported that during chick and mouse gastrulation, cell fates are flexible. When epiblast fragments corresponding to prospective chick somitic mesoderm and heart cells are exchanged during mid- to late-primitive streak stages, cells assume the fate of their new location. This developmental potential is reduced as cells ingress through the primitive streak, and the competence to form somitic cells appears at the mid-streak stage (G. Schoenwolf, P. Tam). Gastrulation orchestrates considerable cell movements in the posterior primitive streak. However, C. Stern (Columbia University, NY) reported that cell movements in the PSM appear to be severely limited, and complete disruption of the rostrocaudal pattern by dissociation of these cells results in their failure to sort out to form normal somites.

Cell lineage analysis using vital dyes has been instrumental in determining the cellular origins of embryonic tissues. These studies have demonstrated that domains constituting the presumptive notochord, medial and lateral somite moieties and lateral mesoderm can be topographically mapped in the primitive streak (C. Stern, G. Schoenwolf). In spite of this apparent geographic address, experimental rotation of the immature somite results in cells adopting the fate of their new position, therefore somite cell fate is also governed by local environmental signals. However, polarity is already established in the PSM since its rotation along the rostrocaudal axis results in somites with a reversed pattern of neural crest cell migration (see below). In addition, prospective somite units in the PSM are encoded with positional information by the Hox code. Indeed, transplantation of thoracic level PSM to the cervical region results in rib formation rostrally (see Keynes and Stern, 1988). How, and at what stage is this Hox pattern established and propagated? This remains unclear since the insertion of physical barriers in the early embryo does not prevent rostral propagation of Hox patterning signals (S. Gaunt, Babraham Institute, United Kingdom).

J. F. Nicolas (Institut Pasteur, Paris) reported on an elegant genetic approach to cell lineage studies in transgenic mice (Nicolas et al., 1996). A muscle promoter linked to a *laacZ* (internal duplication; inactive) reporter gene, undergoes a random intramolecular recombination event (active *lacZ*; optionally triggered by Mitomycin C in utero) and marks the myotomal descendants of a clone. This retrospective analysis predicts that 100–150 cells constitute the myotomal stem cell population in the PSM and that somites form with a calculated periodicity. This is in good agreement with lineage studies in chick using vital dyes (128 cells; C. Stern).

M. George-Weinstein (Philadelphia College of Osteopathic Medicine, PA) raised the possibility that in the PSM and epithelial somites, "founder cells" may recruit uncommitted cells into myogenesis, a process stimulated by the cell adhesion molecule N−cadherin. In

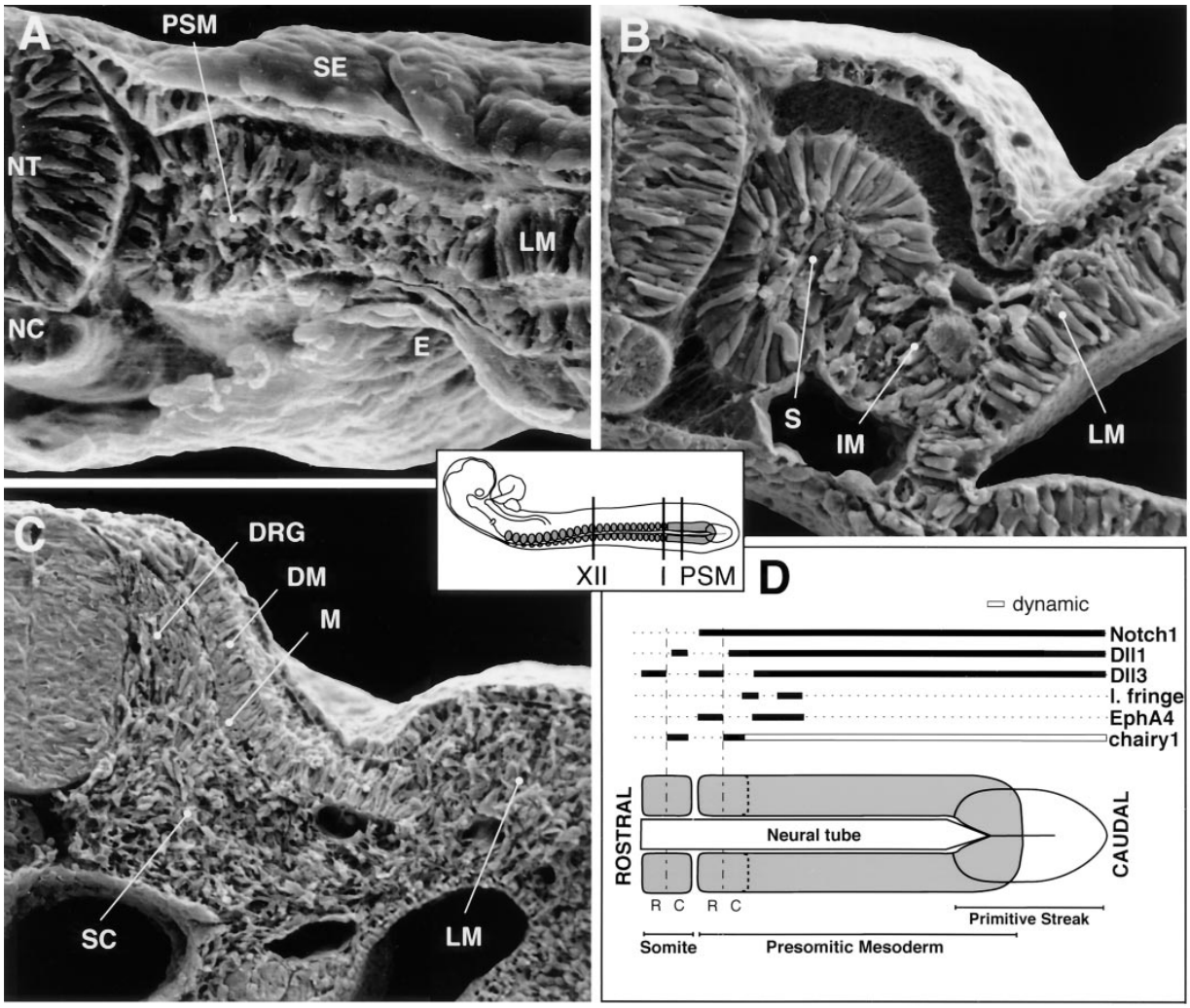

Figure 1. Scanning Electron Micrographs and Schema Portraying Somite Development

The schematic (center) indicates relative maturity at each axial level. A chick embryo is illlustrated for convenience due to development via a flat germinal disc, whereas mouse embryos exhibit a curvature. Somites advance through each stage indicated by roman numerals (Christ and Ordahl, 1995), as seen from transverse views: (A) mesenchymal presomitic mesoderm (PSM). (B) somite I, most recently formed epithelial somite (S) containing a mesenchymal core (somitocoele). An extracellular matrix and basement membrane surrounds the immature somite except for its connection to the intermediate mesoderm (IM; Christ and Ordahl, 1995). (C) Mature stage XII somite (shown from hindlimb level, therefore the hypaxial somitic bud is reduced, see text), dermomyotome (DM), myotome (M), and sclerotome (SC) are distinguishable. (D) Approximate expression patterns (mouse E8.5–9.5) indicate metameric expression prefiguring somites (see text). The most recently forming somite (somitomere) in the rostral PSM is indicated. The chick PSM is approximately double the length of that of a mouse embryo at a comparable stage. Gastrulation takes place in the primitive streak, which initially forms by a thickening of the epiblast cell layer. See Johnston et al. (1997) for *lunatic fringe* expression. *Dll1* expression persists in all somites while *Dll3* appears only in the caudal-most somites (Dunwoodie et al., 1997). *EphA4*, formerly *Sek1* (Orioli and Klein, 1997). C, caudal; DRG, dorsal root ganglion; E, endoderm; LM, lateral mesoderm; NC, notochord; NT, neural tube; R, rostral; SE, surface ectoderm. Electron micrographs (A–C) courtesy of K. Tosney.

contrast, when epiblasts (prior to gastrulation) are dissociated into single cells in serum-free medium, predominantly muscle cells are observed. This competence is repressed when epiblasts are cultured as intact tissue (M. George-Weinstein). Therefore cell–cell interactions may also be necessary to inhibit differentiation in vivo. Interestingly, the Notch-Delta signaling pathway implicated in selecting distinct cell fates from a group of equivalent cells (lateral inhibition) in *Drosophila*, has also been shown to repress myogenesis (see Cossu et al., 1996). Importantly, these genes are strongly expressed in the primitive streak and PSM (Figure 1D). Therefore, uncoupling of a pathway necessitating cell–cell contact may trigger differentiation. Indeed, the phenomenon of lateral inhibition operates in vertebrates since retroviral mediated overexpression of *Delta1* results in ectopic apteria (nude skin) in chick (D. Dhouailly, Institut Albert Bonniot, La Tronche, France).

Somite Segmentation

How are somite segmentation and periodicity defined in the vertebrate embryo? The remarkably dynamic expression pattern of *c-hairy1* in the chick PSM, reported by O. Pourquié, now provides us with some clues. Cyclic waves of expression move rostrally with a periodicity corresponding to the time required to form one somite

(90 min; Figure 1D). This pulsed expression/degradation of *c-hairy1* is an intrinsic property of the PSM and it is not altered by blocking protein synthesis, thus ruling out negative feedback mechanisms implicated in circadian clock rhythms. These experiments suggest that the dynamic *c-hairy1* expression pattern is a read-out of a molecular clock underlying vertebrate segmentation (Palmeirim et al., 1997).

C. Wolff (University of Munich) discussed the possibility of whether a pair-rule code exists in vertebrates. In Zebrafish, the *her1* gene shows a pair-ruled expression pattern (stripes in alternating somite primordia, see citations in Palmeirim et al., 1997), as does the *hairy* gene in alternating segments in *Tribolium* (short germ-band insect) and in *Drosophila* (long germ-band insect). Moreover, like vertebrates, short germ-band insects sequentially add segments from a caudal terminal growth zone. However, *her1* is distantly related to *c-hairy1* and is presently the only vertebrate gene exhibiting a pair-ruled expression pattern (Palmeirim et al., 1997). Therefore, these findings rekindle the debate of whether somitogenesis in vertebrates shares a common ancestry with the segmentation found in insects.

Although the morphological appearance of distinct units (somitomeres) in the PSM remains controversial, a number of developmentally important genes (Figure 1D) clearly exhibit a metameric expression pattern that prefigures somite units in the rostral PSM. Segmentation involves the establishment of boundaries. Recently, Notch, Delta, Serrate, and Fringe have been implicated in specifying the dorsal/ventral boundary in the prospective wing margin of *Drosophila* and chick. Their study in amniotes (ex. chick and mouse), as well as disruption of signaling using components of the Notch pathway in *Xenopus* (C. Kintner, Salk Institute, San Diego, CA), raises the intriguing possibility that they may play a similar role in defining somitomere boundaries in vertebrates. In *Notch1*, *RBP-Jk* (*Drosophila Suppressor of Hairless* homolog; R. Conlon; Case Western Reserve University, Cleveland), and *Delta-like1* (*Dll1*; A. Gossler, Jackson Laboratory, Bar Harbor, ME) null mutant embryos, segmentation is delayed (*Notch1*, *RBP-Jk*) or perturbed (*Dll1*), and somites fail to align across the midline. In these mutants, as well as in *lunatic fringe* null embryos discussed by R. Johnson (Anderson Cancer Center, Houston, TX), somite derivatives are formed suggesting that segmentation and differentiation are separable events. *Dll3*, a divergent Delta homolog, and *Dll1* are expressed mutually exclusively in the rostral-most somitomere of the PSM (S. Dunwoodie, NIMR, London; Dunwoodie et al., 1997). These genes, and *lunatic fringe*, mark distinct subdomains in somitomeres (Figure 1D). In *Dll1* mutants, dorsal root ganglia and myotomes span somite borders, which at first glance suggests problems with segmentation. However, no role for Notch signaling has been demonstrated in invertebrate segmentation. Perhaps Notch signaling in vertebrates defines somitomere boundaries, a necessary step in the segmentation process. Certain Eph family members (Orioli and Klein, 1997) also show metameric expression in somitomeres (Figure 1D) and may thus be candidate molecules for mediating these boundaries. Indeed, expression of a dominant negative form of both ephrin-B2 and EphA4 in early zebrafish embryos results in failure of somite segmentation from the PSM (L. K. Durbin, King's College, London). What factors govern segmentation? Using an in vitro culture system of mouse PSM, R. Conlon discussed the signaling requirements from adjacent structures important for somite segmentation. PSM alone was not competent to segment autonomously and tail bud, but not limb, ectoderm was sufficient to promote segmentation in the PSM. Interestingly, after the last somite has formed, further budding of somites appears to be repressed by the tail bud mesenchyme in vivo (P. Tam).

In *Notch1* and *RBP-Jk* null mutants, epithelialization of somites was suggested to be affected, thereby delaying segmentation (R. Conlon). However, the requirement of epithelialization for segmentation is not absolute since studies with *Paraxis* (basic-HLH transcription factor) null embryos reveals that segmentation, epithelialization, and differentiation are separable events (D. Sosic, University of Texas, Dallas). Indeed, in *Paraxis* null mutants, segmental units corresponding to somites are observed with essentially no epithelial structures, and somite derivatives form, but are disorganized. These findings are consistent with experiments in the chick where the insertion of a barrier between the axial organs, or the surface ectoderm, and the PSM results in down-regulation of paraxis and lack of epithelialization (B. Brand-Saberi, B. Christ, University of Freiburg; D. Sosic).

Overexpression studies in *Xenopus* and targeted gene disruptions have also indicated that a number of other genes may play a role in segmentation. C. Kintner reported that *Thylacine* (*Mesp2* related gene; basic-HLH) overexpression perturbs segmentation but not myotome formation. Consistent with this, Y. Saga (National Institute of Health Sciences, Setagayaku, Japan) reported that in *Mesp2* null embryos, *Notch1* is downregulated and metameric markers (such as *Dll1*) are not expressed segmentally. Similarly, a disruption of sclerotome polarity occurs in *Dll1* mutants (A. Gossler). Therefore, rostral somite halves appear to be respecified to a caudal character suggesting that disturbance of the rostrocaudal polarity results in defective segmentation.

In summary, multiple genes and signaling events are implicated in somite segmentation, a process that designates somite unit length, boundary formation, and an underlying molecular clock regulating the timing of somite output.

Signaling Molecules and Somite Patterning

In the last few years, important signaling molecules have been identified and implicated in the patterning of divergent embryonic structures, including somites. These molecules were a major topic of discussion at the meeting. Somite formation and differentiation depends on signaling molecules released in a coordinated manner from adjacent tissues. As a result, somite derivatives exhibit distinct polarities along the established dorsoventral and mediolateral body axes (see Spörle and Schughart, 1997). Whereas axial structures (neural tube and notochord) and surface ectoderm secrete factors that generally promote regionalization and differentiation via molecules such as the Wnts, Sonic Hedgehog (SHH), and noggin, the lateral mesoderm plays an inhibitory role at least for muscle differentiation, via BMP4 (see

Cossu et al., 1996). The surface ectoderm is required to maintain the dermomyotome as an epithelium (see Christ and Ordahl, 1995). To date, the requirement for signaling from the underlying endoderm has not been extensively investigated. In vivo manipulations and explant culture techniques have become more and more sophisticated, and these are currently the methods of choice for examining how signaling promotes somite regionalization and differentiation. The developmental status of cells following these perturbations is monitored by region- or cell-type-specific markers, many of which are conserved between vertebrate species, suggesting that somite patterning mechanisms are also conserved.

How do the signaling molecules function? P. Beachy (John Hopkins University, Baltimore, MD) discussed important aspects of SHH protein processing that help explain some of its biological activity. An autoproteolytic processing by the C terminus is accompanied by a covalent attachment of cholesterol to the N−terminal fragment. This secreted molecule carries all the biological activity of HH. Cholesterol is implicated in restricting the range of SHH activity, but the use of drugs that block cholesterol synthesis/transport in explants and in vivo, reveals that cholesterol may also mediate the cellular response to SHH signaling.

Although the effective physiological levels of the signaling molecules are not known, pleiotropic effects are clearly observed with different concentrations of SHH. When placed next to the dermomyotome, an ectopic notochord (s), likely via SHH, induces sclerotome at the expense of muscle (see Gossler and Hrabi de Angelis, 1997). However, in grafting experiments, at a distance from a notochord (and in proximity to neural tube or surface ectoderm; S. Dietrich, UMDS Guy's Hospital, London; Borycki et al., 1997; Dietrich et al., 1997), or with SHH-expressing cells or coated beads (C. Emerson, University of Pennsylvania School of Medicine, PA), MyoD is clearly activated. SHH also induces myogenesis in zebrafish (see below). Therefore, SHH thresholds appear to be important for differential activation of sclerotome and muscle, and this may explain some of the reported differences.

Additional roles have been proposed for SHH. N. Le Douarin (IECM, Nogent Sur Marne, France) discussed the importance of axial structures in providing a trophic support to the developing somites; substitution of the neural tube and notochord by SHH-expressing cells rescues somitic cell survival and promotes muscle and sclerotome differentiation. Consistent with this notion, *Shh* null mice activate muscle and sclerotome markers, but at reduced levels (reviewed in Gossler and Hrabi de Angelis, 1997).

Since *Shh* is expressed along the caudal-rostral axis in the notochord and later in the floor plate, it is not clear when SHH acts. To address this point and to distinguish between myogenic induction and maintenance, C. Emerson reported on the regional activation of components of the SHH signaling pathway (see Hammerschmidt et al., 1997). *Patched*, which appears to be an SHH receptor, is not expressed in the PSM but in somite I, suggesting that signaling is first interpreted at this stage. The differential and dynamic expression of *Gli1* and *Gli2/4* (formerly *Gli3*; C. Emerson) leads to the interpretation that both are required for myogenic induction whereas *Gli1* alone plays a role in sclerotome induction. *Gli1* activation depends on SHH whereas *Gli2/4* is activated by the surface ectoderm (and neural tube) independently of SHH. Reduced SHH levels result in the down-regulation of *Patched*, thus revealing the feedback nature of this signaling.

If SHH is necessary for induction of somite differentiation, Wnts are clearly also implicated subsequently. Indeed cooperation with SHH to induce myogenesis in avians has been demonstrated for Wnts 1, 3a, and 4 (Münsterberg et al., 1995) and Wnts have mitogenic activity in vitro (see Cossu et al., 1996). For the mouse, G. Cossu (University of Rome) provided evidence that different Wnts may have differential effects on the muscle determination genes *Myf5* and *MyoD* since Wnt 7a preferentially activates *MyoD* whereas Wnt 1 preferentially activates *Myf5*. However, it is unclear which Wnts act in vivo. G. Cossu also discussed putative repressors of myogenesis. *Frzb* (secreted Wnt receptor), expressed in the mesenchyme adjacent to somites, may sequester Wnts and limit their action. However, unravelling these interactions appears daunting since numerous Wnt, frizzled (presumptive Wnt receptors), and Frzb molecules have been identified.

Unlike the vertebrate clades, in urochordates (ascidians) where segmentation and somites are not clearly evident, maternal muscle cell determinants are already localized in the fertilized egg and precede the activation of a *MyoD*-related gene in the early embryo, as reported by J. Chenevert (CNRS, Observatoire de Villefranche, France).

BMP4 acts as a negative regulator of somite diffentiation and, like SHH, in a dose-dependent fashion. Administration of high levels of BMP4 to somite explants (cocultured with surface ectoderm), blocks both *Pax3* and *MyoD* expression. Interestingly, intermediate BMP4 levels block *MyoD* but not *Pax3* expression, and lower levels result in the activation of both genes. Therefore, differences in BMP levels may modulate whether ectodermal signals activate solely dermomyotomal markers (*Pax3*) or both dermomyotomal and myotomal genes (R. Reshef, Harvard Medical School, Boston, MA). Surprisingly, elevated BMP4 levels can convert PSM to lateral mesoderm (Y. Takahashi, Kitasato University, Japan; Tonegawa et al., 1997).

In the embryo, the effective levels of BMP4 could be regulated either by modulating gene expression, or by antagonism of BMP4 protein via proteins such as noggin (E. Hirsinger, Developmental Biology Institute of Marseille; C. Marcelle, Caltech, Pasadena, CA; R. Reshef). BMP4 expression is high in the lateral mesoderm. Intriguingly, in immature caudal somites and PSM, noggin transcripts are detected in the lateral moieties apposed to the BMP4 signal, whereas in more mature rostral somites, noggin expression shifts medially adjacent to the neural tube (E. Hirsinger, C. Marcelle, R. Reshef). Remarkably, when *noggin*-expressing cells were placed in the prospective lateral mesoderm at the level of the primitive streak - supernumary somites (up to three) formed in a mediolateral direction (Y. Takahashi). Therefore, the antagonism of BMP4 by noggin appears to be important in defining tissue borders as well as preventing premature differentiation. SHH secreted by the

notochord may regulate *noggin* expression since SHH-expressing cells can induce ectopic *noggin* (E. Hirsinger). Moreover, *Patched* expression in the epaxial dermomyotome lip (C. Emerson) suggests that SHH here may activate *noggin*.

In addition to its lateral expression, BMP4 is expressed in the dorsal neural tube region. C. Marcelle reported that in chick, BMP4 in the dorsal neural tube indirectly induces formation of the epaxial dermomyotome lip via activation of Wnt 1 and 3a in the neural tube. In addition, Wnt signals from the neural tube can promote *noggin* expression in the epaxial dermomyotome lip (E. Hirsinger), suggesting that here too noggin may limit BMP4 action.

It has recently become apparent from work in *Xenopus* that BMP4 acts as a ventralizing signal that is antagonized by dorsally expressed *noggin* (or *chordin*) to promote dorsal cell fates (Graff, 1997). M. Halpern (Carnegie Institution, Baltimore, MD) presented genetic evidence that ventralizing signals must be antagonized for normal patterning of the zebrafish embryo and hence of somites. Indeed, mutations of *chordin* result in reduced neuroectoderm and somites and increased ventral mesoderm.

It is clear that "naive" somitic cells are patterned by local environmental signals. However the somite, classically considered to be a responding tissue, itself also has inducing capabilities on the neuroectoderm. R. Krumlauf (NIMR, London) reported that when caudal somites were transplanted rostrally (hindbrain level), they were capable of extending Hox expression in the adjacent neuroepithelium more rostrally. This phenomenon was monitored in an in vitro reconstitution assay with rhombomeres and somites. A novel protein identified from an expression library generated from somite mRNA is able to mediate some of the required activities. In a related study, C. Lance-Jones (University of Pittsburg, PA) reported that local signals from the PSM play a role in stabilizing *Hox* gene expression patterns within the spinal cord.

Somites are polarized along the rostrocaudal axis and thereby influence neural crest migration. Neural crest cells emanating from the dorsal neural tube follow two migration routes: either dorsally between the dermomyotome and surface ectoderm, or ventrally through the rostral, but not caudal, sclerotome. M. Bronner-Fraser (Caltech, Pasadena, CA) discussed this ventral migration route and the role of Eph-related molecules in the context of the permissive (rostral) and repulsive (caudal) character of the sclerotome. Using exogenous ligand as a competitive inhibitor in a three-dimensional explant system, ephrin-B1 ligand produced by the somite was repulsive to neural crest migration, thus implicating this class of molecules in their guidance.

The use of chick/quail chimeric grafts has been instrumental in investigating somite development, and recently the use of mouse/chick chimeric grafts has expanded on this technology. J. Fontaine-Pérus (Faculté des Sciences et des Techniques, Nantes, France) reported that in mouse/chick somitic chimera, mouse muscle progenitors contribute to chick epaxial and hypaxial muscles. This approach now permits the recombination of genetically modified mouse tissues with that of chick, and the subsequent analysis in a developmental context.

Somite Subdivisions and Differentiation

Studies in avians have demonstrated that in addition to muscle, sclerotome, and epaxial dermis, somites give rise to connective tissue and blood vessels (see Christ and Ordahl, 1995). Therefore, somite derivatives provide not only the vertebrate body scaffold, but also the associated tissues for this structure to function. Differentiation of dermis and the other somite derivatives, however, remains relatively poorly understood. In contrast, myogenesis has served as a standard for cell fate determination and differentiation since determination genes for this lineage have been identified and well-defined culture systems are available. C. Ordahl (University of California, San Francisco) discussed attempts to define determination in chick somite derivatives by challenging epaxial myotome progenitor cells from the dorsomedial quadrant of somites of different developmental ages with ectopically positioned notochords. Myotome progenitor cell fate became progressively restricted in a developmental time frame roughly correlating with the onset of *MyoD* and *Myf5* expression.

Somite derivatives can be subdivided into epaxial and hypaxial domains based on their differing signaling requirements as well as anatomical criteria (Figure 2; see Spörle and Schughart, 1997). Epaxial myogenic induction (adjacent to neural tube) is dependent on axial structures whereas hypaxial (adjacent to lateral mesoderm) myogenesis is under the influence of surface ectoderm and lateral mesoderm (see Cossu et al., 1996). This subdivision is clearer for myotome formation, whereas sclerotome subdomains remain to be better defined. All skeletal muscle in the trunk, tail, and limbs (and some head muscles) originate from somites.

The spatiotemporal origin of muscle progenitor cells from the dermomyotome has been debated for over a century. During this period, the field has evolved from careful morphological observations to sophisticated microsurgical and molecular genetic manipulations of embryos. These diverse approaches in chick including vital dye injections (K. Tosney, University of Michigan, Ann Arbor), in combination with confocal imaging (W. Denetclaw, University of California, San Francisco; Denetclaw et al., 1997), quail/chick chimeras (K. Tosney), and cell proliferation assays (C. Kalcheim, Hebrew University of Jerusalem-Hadassah), together with observations of developmentally arrested progenitors in Myf5nlacZ knock-in mice (S. Tajbakhsh), have partly resolved this controversy. Myogenic progenitors were reported to arise initially in the medial epithelial somite, with subsequent contributions from the dermomyotome, mainly from its epaxial and hypaxial edges and to a lesser extent, the rostral and caudal edges and its sheet. Some reported differences in findings appeared to lie in somite age, position (limb versus interlimb), and the species analyzed. K. Tosney reported that even experimentally induced cuts producing a CAP (center of active progenitors) would produce muscle cells, supporting the idea that all of the dermomyotome edges are sites of muscle cell production. Other evidence for subdivisions within

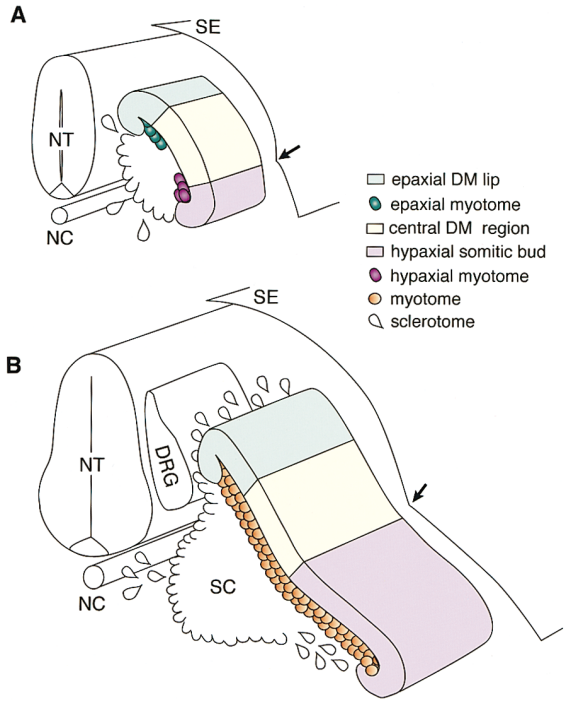

Figure 2. Schema of Differentiating Mouse Interlimb Somite

(A) Muscle progenitor cells originate from epaxial and hypaxial dermomyotome domains (about E9.5).

(B) Somite derivatives elongate along the dorsoventral body axis. Cells from the hypaxial (constitutes the bulk of muscle in amniotes) and epaxial myotomal subregions form the continuous myotome layer (about E10.5). The epaxial and hypaxial dermomyotomal extremities remain epithelial longest, while the central dermomyotome (marked by *En1* and *Sim1* expression) becomes mesenchymal first, contributing to dermis and myocytes (see text). Arrows indicate indentation in the body wall, a morphological landmark. Abbreviations are as in Figure 1.

the myotome came from P. Rigby (NIMR, London) who presented a detailed analysis of *Myf5* regulatory sequences that revealed multiple enhancers for expression in epaxial and hypaxial compartments, and different hypaxial subdomains. Consistent with the idea that progenitors reside within the dermomyotome, this structure contains proliferating cells, whereas the myotome has until presently been viewed as a postmitotic compartment (Christ and Ordahl, 1995). Surprisingly, however, C. Kalcheim proposed that an initial population of myotomal "pioneer" cells (postmitotic) serves as a scaffold for future myotomal cells that are derived from proliferating progenitors in the dermomyotome lips, and later within the myotomes themselves.

Intercalated between the epaxial lip and hypaxial somitic bud, a third central population of muscle progenitors was discussed (P. Rigby, K. Schughart, Transgene SA, Strasbourg, S. Tajbakhsh, K. Tosney; Figure 2). They can be specifically marked by *En1* (*engrailed 1*) and *Sim1* (*Drosophila single minded* homolog) expression (K. Schughart, K. Tosney), and they give rise to myocytes as the central dermomyotome forms dermis (K. Tosney). Analysis of *Pax3* (*splotch*) mutant embryos (see below) demonstrated that Pax3 is not necessary for specifying these cells (S. Tajbakhsh).

The possible intercalation of a central domain of *En*-expressing cells between more epaxial and hypaxial myotomal subdomains in amniotes raised the intriguing possibility that the organization of these subdomains may have been conserved among amniotes and zebrafish (K. Schughart). In zebrafish, these subdivisions and their signaling requirements have been somewhat more clearly defined; slow muscle progenitor (adaxial) cells abut the notochord and can be distinguished from the more lateral fast muscle progenitors. M. Westerfield (University of Oregon, Eugene) reported that adaxial slow muscle progenitors either migrate away radially to form the superficial layer of the myotome, or they remain (in the adaxial region) to form *En*-expressing muscle pioneers intercalated between epaxial and hypaxial myotomal domains. Interestingly, *you-type* zebrafish mutants (van Eeden et al., 1996) exhibit defects of muscle pioneers and the horizontal myoseptum, which precisely divides epaxial and hypaxial myotomal domains (P. Haffter, MPI of Developmental Biology, Tübingen).

Unlike amniotes, the three *hh* homologs in zebrafish are expressed in the notochord and/or floor plate and appear to have differential abilities in inducing slow muscles (Blagden et al., 1997; Du et al., 1997). In ectopic expression assays, SHH and dominant negative PKA (negative regulator of SHH signaling) can convert the predominantly fast myotome to slow muscle (M. Westerfield, P. Currie, ICRF, London). Additionally, ectopic expression of *dorsalin* (TGFβ factor; see BMP4 in the previous section) in the notochord blocks muscle pioneer formation from the adaxial region, suggesting that BMP−like inhibitory signals act in epaxial and hypaxial myotomes (M. Westerfield). Therefore, the identification of three different muscle-forming regions in zebrafish (epaxial, adaxial, hypaxial), and the specific requirement of adaxial cells for HH signaling may give deepened insights into the relevance of myotomal subdomains in amniotes. These subdomains might be considered in relation to the topographical changes during neural tube infolding which internalized the original dorsal pole, the notochord, into the depth of the vertebrate embryo (Spörle and Schughart, 1997). In another study, P. Haffter reported that in one *you-type* mutant, *sonic you*, the *Shh* gene was deleted, and the floor plate was present in the absence of a notochord. This surprising finding suggests that in zebrafish, SHH is not required to induce floor plate, but rather plays a role in the induction of the adaxial cells and cells lateral to the floor plate in the ventral neural tube.

In fish and amphibians, muscle differentiates very early and forms the bulk of the early somite, whereas in amniotes, sclerotome differentiates first. This perhaps reflects their respective life strategies where nonamniote larvae must respond rapidly in a hostile environment whereas amniote in utero development proceeds further before their release into full gravity. In amniotes, the epithelial dermomyotome may serve an organizing role for myotome regionalization and delaying myogenesis. Indeed, in mouse mutants exhibiting somite epithelialization defects (discussed above), myotomal and sclerotomal derivatives, at least in part, are present but disorganized.

Several speakers addressed the topic of somite development and differentiation using mouse mutants. *Mox1*

and *Mox2* (mesoderm-mesenchyme homeobox genes) were reported by B. Mankoo (NIMR, London) to act as key players in somite cell differentiation programs. Whereas mice carrying mutations in *Mox1* or *Mox2* display sclerotome and muscle abnormalities, respectively, in *Mox1/Mox2* double mutant mice, *Pax1*, *Pax9*, *Pax3*, *Pax7*, and *twist* expression were not detected or were severely reduced in somites. Consequently, the axial skeleton and ribs were absent and severe muscle defects were evident (see also below). Indeed, R. Balling (GSF-Research Center, Neuherberg, Germany) reported that *Pax1/Pax9* double mutants suggest redundance between *Pax1* and *Pax9* for axial skeleton development. Although *Pax9* expression in sclerotome domains only partly overlaps with that of *Pax1*, *Pax9* null mutants do not exhibit obvious axial skeletal defects. In contrast, a targeted null allele of *Pax1* reproduced the phenotype of *undulated* mutant mice with semidominant defects of the trunk axial skeleton (R. Balling).

Sclerotome development and chondrogenesis during chick embryogenesis were also discussed. In the keynote lecture, B. Christ presented a historical perspective of the field and focused on the controversial topic of resegmentation: does a single somite contribute to one or two vertebral segments? Although the classical data has been conflicting (Keynes and Stern, 1988), in chick/quail chimeric grafts, a single somite can contribute to two vertebrae and ribs (Huang et al., 1996). In other studies with chick/quail chimeras and labeling of adjacent somites with fluorescent dyes, in the majority but not all cases, resegmentation was not observed (C. Stern). This issue therefore remains unresolved. Nevertheless, around the axial organs two different chondrogenic mesenchymes can be detected: chondrification of the vertebral body and neural arches is Pax1-mediated, whereas dorsal to the neural tube, chondrogenesis of the spinous processes is Bmp4- and Msx1/2-mediated (N. Le Douarin). Interestingly, ribs also originate from two different mesenchymes. This point was discussed by H. Koseki (Chiba University, Japan) who reported on barrier experiments in chicken revealing two different sources of rib forming cells: vertebral versus sternal.

In contrast to the epaxial and hypaxial trunk myotomes, hypaxial limb muscle progenitors migrate into the limb bud as mesenchymal cells and are marked by *Pax3* and *c−met* (tyrosine kinase receptor) expression. Limb skeletal elements, however, are derived from the lateral mesoderm, not somites. It is noteworthy that hypaxial somitic buds mature from dermomyotomes in interlimb somites, but limb tissue prevents their maturation by inducing apoptosis in limb somites (K. Tosney; Figure 1C). New markers for limb muscle progenitors, *Lbx1* (*Drosophila ladybird* homolog; S. Dietrich, M. Goulding, Salk Institute, La Jolla, CA) and *26M15* (Sp1 related gene; S. Dunwoodie) were also discussed. *Lbx1* expression is present in the trunk of *c-met* null embryos but absent in *splotch* mice. E. M. Füchtbauer (MPI of Immunobiology, Freiburg) reported on *twist* (important for *Drosophila* mesoderm formation) which is an antagonist of myogenesis. Unexpectedly, in *twist* null embryos myoblasts at the limb level fail to leave the somite, while epithelial migration at the interlimb level appears to be normal.

In *splotch* mutant embryos, the hypaxial somitic bud is severely reduced and epaxial, hypaxial, and notably limb muscle deficiencies are observed. A. Mansouri (MPI of Biophysical Chemistry, Göttingen) reported that *Pax7* null embryos do not have any apparent somitic defects, however in *Pax3(splotch)/Pax7* double mutant embryos, the dermomyotome is severely reduced in size or absent, but *Myf5* is expressed and myogenesis proceeds. This finding is consistent with the phenotype of *splotch/Myf5nlacZ* double mutant embryos where *Pax3* and *Myf5* were found to act genetically upstream of *MyoD* and the former two act in parallel genetic pathways (Tajbakhsh et al., 1997). Although *Pax3* is thought to act genetically upstream of *c-met* for skeletal myogenesis, preliminary analysis of *c-met*$^{-/-}$ *Myf5nlacZ*$^{+/-}$ mutants (S. Tajbakhsh) revealed that at least for some abdominal muscles, c-met is not the sole mediator of Pax3 function.

Conclusions

Somitogenesis requires the orchestration of multiple events including somite unit definition, periodicity, and segmentation. In addition, signaling between adjacent structures and paraxial mesoderm allows somitogenesis to proceed, and vice versa for patterning of the neural tube and its derivatives. Numerous universal signaling molecules have now been identified, along with their effects in governing somite formation and differentiation; this has permitted the first glimpse of how cell fate in multiple tissue types is established. An important challenge will be determining how these signaling cascades are interpreted to effect morphogenetic movements of tissues and to confer identity. Unravelling the details should help us decipher the many developmental defects associated with somite derivatives, and comprehend how the modular organism is formulated into a coherent structure.

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