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The formation of somite compartments in the avian embryo

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ABSTRACT The somites develop from the unsegmented paraxial mesoderm that flanks the neural tube. They form in an intrinsic process which lays down the primary segmental pattern of the vertebrate body. We review the processes of somitogenesis and somite differentiation as well as the mechanisms involved in these developmental events. Long before overt differentiation occurs, different compartments of the still epithelial somites give rise to special cell lines and to particular derivatives. By means of isotypic grafting between quail and chick embryos, it is possible to follow the fate of groups of somitic cells. In this way, the development of the myotome and the back dermis from the dorsomedial quadrant and of the hypaxial body wall and limb musculature from the dorsolateral quadrant was established. The two ventral quadrants and the somitocoele give rise to the chondrogenic/fibroblastic lineage of the sclerotome and form the vertebral column. Somite compartments can first be visualized by the expression pattern of *Pax* genes. *Pax-3* is expressed in the dorsal part of the epithelial somite, while the ventral two thirds express *Pax-1*, a marker of sclerotome development. *Pax-3* expression is retained also in the premitotic myogenic cells that migrate into the limb buds. In differentiating myoblasts, *Pax-3* expression is turned down and taken over by the activation of MDF's. This initial event in myogenesis occurs in the absence of local signals, whereas the expression of *Pax-1* in the sclerotome can be shown to be induced by signals from the notochord and floor-plate of the neural tube. Epaxial myotome differentiation is supported by the neural tube, after the neural tube has received patterning signals from the notochord. The hypaxial musculature and limb musculature differentiate independently of the axial structures. The myogenic cells migrating within the limb buds respond to signals of the lateral plate mesoderm which guide their distalward migration and pattern the muscle.

KEY WORDS: *somite, Pax, MDFs, myogenesis, avian embryo*

Introduction

The somites are the most original and at the same time the most characteristic structures of the vertebrate embryo. The former attribute holds true because they impose a primary segmented pattern on the vertebrate body – a feature shared by its ancestors, and the latter is reflected by the fact that the somites give rise to the structures characterizing the whole taxonomic group: the vertebrae.

The acquisition of an efficient motoric system including the evolution of paired appendages was only possible by the elaboration of a body pattern functionally combining stabilizing and contracting elements: the vertebral column and the originally also segmented system of skeletal muscle (Keynes and Stern, 1988; Christ *et al.*, 1990; Christ and Wilting, 1992; Wachtler and Christ, 1992). The significance of the somites for the evolution and functional morphology of the vertebrates is undeniable and might by itself justify the intense study of this developmental speciality by the biologist and medical scientist, especially since many congenital defects originate from various problems during somite development. Moreover, the somites fascinate the developmental biologist because they are examples of and allow the examination of nearly all major mechanisms involved in embryogenesis such as epithelio-mesenchymal transformation, cell-cell-signalling, cell-matrix interactions, cell migration and cell differentiation.

The somites as segmentation elements possess definite boundaries delimiting each unit and, during further development, their cells give rise to daughter cells that are also restricted to these boundaries. Together with a spatially restricted gene expression, these factors characterize the somites as developmental compartments (Stern *et al.*, 1986; Lawrence, 1990). More recently, investigations have shown that the early somite already consists of different regions giving rise to particular derivatives in

Abbreviations used in this paper: CSPG-6, chondroitin-6-sulphate-proteoglycan; ECM, extracellular matrix; MDFs myogenic determination factors; PNA, peanut agglutinin.

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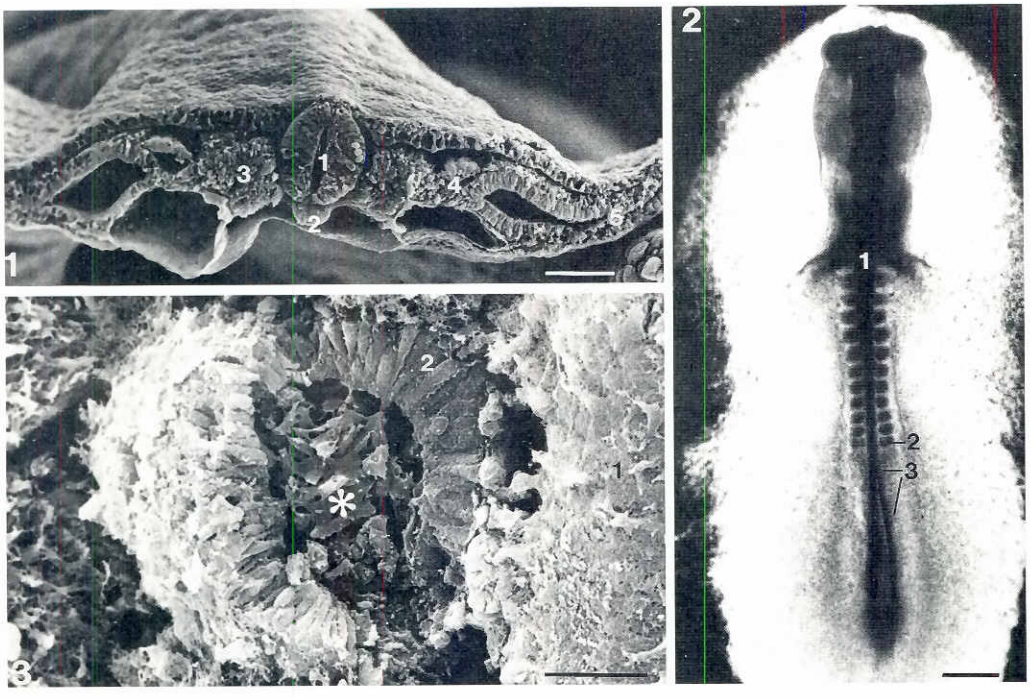


Fig. 1. Transverse fracture of a stage 16 HH chick embryo at the level of the forelimb buds. *Scanning EM view towards the cranial end. 1 neural tube, 2 notochord, 3 somite, 4 intermediate mesoderm, 5 lateral plate mesoderm. Bar, 100 µm.*

Fig. 2. Dorsal aspect of a chick embryo at two days of incubation. *The sequence of somites shows a cranio-caudal gradient of differentiation with the most advanced stages at the cranial end, and still unsegmented paraxial mesoderm at the caudal end. 1 neural tube, 2 last somite, 3 unsegmented paraxial mesoderm. Bar, 500 µm.*

Fig. 3. Scanning EM view of an epithelial somite of a chick embryo at stage 16 HH. *1 neural tube, 2 somitic epithelium, * somitocoele. Bar, 25 µm.*

permissive conditions (Christ *et al.*, 1992; Ordahl and Le Douarin, 1992; Brand-Saberi *et al.*, 1994; Huang *et al.*, 1994; Wilting *et al.*, 1995) and that during somite differentiation the pattern of genes (Goulding *et al.*, 1994; Williams and Ordahl, 1994; Ebensperger *et al.*, 1995; Neubüser *et al.*, 1995) delineates compartments within the somite. The process of compartmentalization is the first event in somite regionalization and represents the prerequisite for differentiation. The idea of somitic compartments explains the way in which the somites not only serve as sources but also as distributors of their own derivatives. Since this term was first applied to the avian somite and most of our combined efforts as well as those of other groups were aimed at this system, aspects of avian somitogenesis will be focused in this review. We will highlight the processes of myogenesis, sclerotome formation, and cell migration into the limb buds on the basis of our work involving microsurgical manipulations such as quail-chick chimeras (Le Douarin, 1969) and other experimental *in ovo* approaches. Problems of signal transduction, cell lineage and directed cell migration in connection with developmental mutants of the mouse will also be addressed.

Somitogenesis

After gastrulation, mesodermal derivatives can be subdivided topographically into five groups: the notochord, the paraxial mesoderm, the intermediate mesoderm, the lateral plate and the prechordal mesoderm of the head region. The origin of the paraxial mesoderm from which the somites form, is still a matter of controversy. According to Pasteels (1937) and Selleck and Stern (1991) the medial and lateral portion of the paraxial mesoderm derive from different populations of gastrulating cells, whereby the medial half comes from cells passing through Hensen's node, whereas the lateral half derives from cells passing through the cranial primitive streak. In contrast to this opinion, Schoenwolf and co-workers (1992) were unable to detect contributions from Hensen's node. In any case, gastrulation is the event that renders cells from the epiblast competent to differentiate into skeletal muscle in permissive conditions (Krenn *et al.*, 1988).

The paraxial mesoderm is located between the intermediate mesoderm and the neural tube, its ventromedial portion borders on the notochord (Fig. 1). The somites detach from the highly proliferative cranial end of the unsegmented paraxial mesoderm by compaction and epithelialization (Lash *et al.*, 1984; Stern and Bellairs, 1984). The products of somitogenesis are epithelial pseudostratified spherules, the outside of which is covered by a basement membrane (Solursh *et al.*, 1979), and the lumen (somitocoele) of which is occupied by mesenchymal cells (Fig. 3). The process of somite formation proceeds from cranial to caudal levels and the sequence of somites reflects a cranio-caudal gradient of developmental progress (Fig. 2). The number of somites in an avian embryo has been used by Hamburger and Hamilton (1951) as part of their stage classification system. Since the developmental status of each pair of somites differs along the cranio-caudal axis, a staging system has recently been introduced to characterize each somite pair by its own developmental stage (Ordahl, 1993; Christ and Ordahl, 1995).

The process of segmentation is an inherent capacity of the paraxial mesoderm, since it undergoes segmentation in isolation from all neighbouring structures. This means that the process is not controlled by extrinsic signals (Bellairs, 1963, 1979; Christ *et al.*, 1972, 1973; Menkes and Sandor, 1977; Packard, 1978). Moreover, the paraxial mesoderm conveys segmentation to other tissues such as the nervous system and the blood vessels (Christ, 1975; Rickman *et al.*, 1985; Stern *et al.*, 1986). The further differentiation of the somites is characterized by the segregation of different cell lineages giving rise to two groups of skeletal muscle (epaxial and hypaxial), to cartilage, bone and

connective tissue of the vertebral column, to connective tissue of the dorsal skin, and to endothelial cells and smooth muscle (Christ and Jacob, 1980; Wachtler *et al.*, 1981; Brand *et al.*, 1985; Solursh *et al.*, 1987; Lance-Jones, 1988; Schramm and Solursh, 1990; Noden, 1991; Brand-Saberi *et al.*, 1994; Wilting *et al.*, 1995).

Somite compartments

By means of homotopic interspecific grafting, it was shown that the different quadrants of the unsegmented paraxial mesoderm and of the epithelial somite give rise to particular structures of the embryonic body (Fig. 4). Both chondrogenic and myogenic lineages comprise subdivisions of cells that can be attributed to individual somitic compartments (cartilage, connective tissue or muscle) and give rise to distinct derivatives. In this way, the dorsomedial quadrant alone differentiates into the epaxial intrinsic back muscle and the dorsolateral quadrant contains the precursor cells of the hypaxial muscle of the ventral body wall, and the skeletal muscles of the limbs (Ordahl and Le Douarin, 1992). In contrast to the dorsolateral quadrant, the dorsomedial quadrant also gives rise to the connective tissue surrounding the muscle groups that are derived from it. The connective tissue of the limb muscles originates from the lateral plate mesoderm (Christ *et al.*, 1977). The dorsomedial quadrant also gives rise to the back dermis through the dermomyotome. Thus the dorsal half of the epithelial somite (and the still unsegmented paraxial mesoderm) is dedicated to the myogenic, fibrogenic and angioblastic lineages, whereas the two ventral quadrants yield material of the vertebral column including the intervertebral discs and parts of the ribs. Both structures receive contributions from the somitocoelic cells (Huang *et al.*, 1994). The ventromedial quadrant contains the chondrogenic precursor cells of the vertebral body, pedicle and proximal rib (rib homolog, respectively), whereas the ventrolateral quadrant yields the main portion of the ribs. Regarding its participation in the formation of particular body structures, as was demonstrated by orthotopic grafting (Huang *et al.*, 1994), the somitocoele must be counted as a separate compartment.

Regarding the cranio-caudal axis, several studies show that somitic compartments can also be distinguished here (Keynes and Stern, 1984; Ranscht and Bronner-Fraser, 1991). Only the cells in the caudal half of the somite possess peanut agglutinin (PNA) receptors (Keynes and Stern, 1984). Neural crest cells and axons only invade the cranial half of the epithelial somite and later migrate through the cranial sclerotome. At the stage of sclerotome formation, the cranial portion can be distinguished from the caudal portion also by differences in proliferation indices and the distribution of chondroitin-6-sulphate (CSPG-6), tenascin, and versican (Wilting *et al.*, 1994, Landolt *et al.*, 1995; Fig. 5, 6). CSPG-6, PNA-receptors, and versican in the caudal half are thought to act as repellents for neural crest cell and axon invasion (Stern *et al.*, 1986; Oakley and Tosney, 1991; Landolt *et al.*, 1995).

The basic helix-loop-helix gene *twist* has been found to be strongly expressed in the caudal half of the sclerotome (Füchtbauer, 1995).

Differentiation

Differentiation can be defined at the biochemical level as the diversification of precursor cells by the accumulation of tissue-specific proteins (luxury molecules; Grant, 1978) and at a histological level referring to the arrangement of cells. Moreover, since it has been possible to visualize the developmental bias of a cell at the level of gene transcription, one might argue that another -transient- kind of differentiation precedes the two aforementioned ones: the expression of control genes. In the somites these are genes of the *Hox*-, *Pax*- and the basic helix-loop-helix (bHLH) transcription factor family.

Hox-gene expression is involved in regional specification of the somite sequence (Kessel, 1991; Kessel and Gruss, 1991), whereas *Pax*-genes appear to play a role in the early differentiation of each individual somite. The unsegmented paraxial

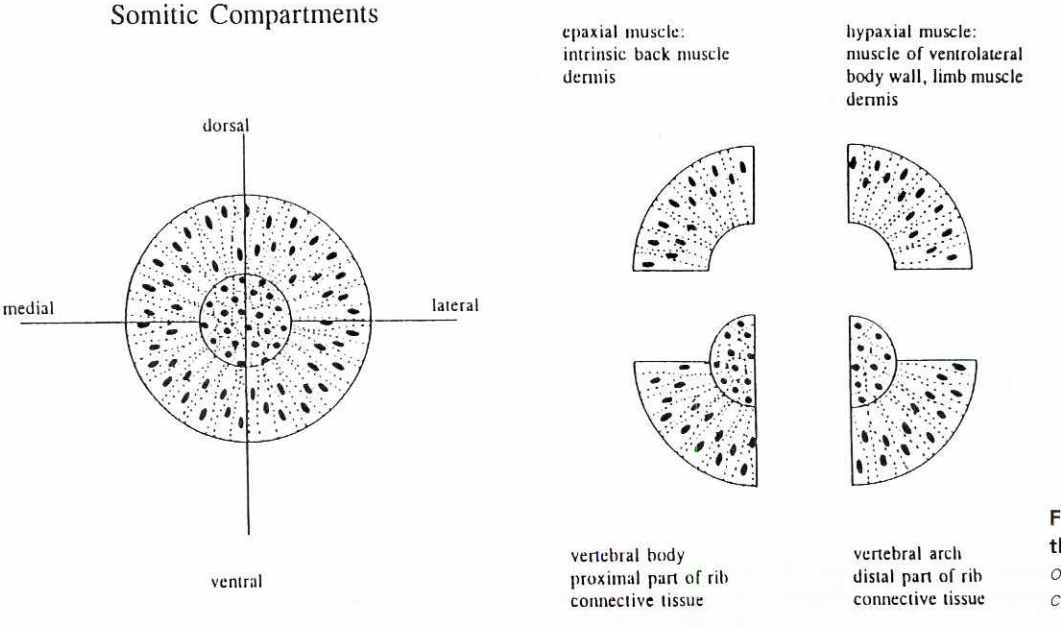


Fig. 4. Somitic compartments and their derivatives. *Cells in the centre of the epithelial somite are somitocoele cells.*

mesoderm expresses *Pax-3* and *Pax-7* (Bober *et al.*, 1994; Goulding *et al.*, 1994; Williams and Ordahl, 1994).

After the somites have formed, the expression of *Pax-3* becomes restricted to the dorsal third, while the ventral two thirds and the somitocoele accumulate *Pax-1* and *Pax-9*-transcripts (Goulding *et al.*, 1994; Williams and Ordahl, 1994; Ebensperger *et al.*, 1995 Neubüser *et al.*, 1995). As we will discuss in the following chapter, these changes depend on local signalling (cell-cell interactions or diffusible factors). Thus, *Pax*-gene expression in the epithelial somite is closely correlated with the future fate of its compartments: The ventral *Pax-1*-positive portion of the epithelial somite breaks up into mesenchymal cells which form the sclerotome, whereas the most dorsal *Pax-3*-positive portion retains its epithelial character and forms the dermomyotome (Fig. 7). *Pax-1* expression is initiated in the third somite from caudal (Fig. 8).

Through the lateral folding of the embryo, the ventral somite derivative – the sclerotome – becomes shifted medially and the dorsal derivative – the dermomyotome – comes to lie most laterally (Fig. 9). The cells located in the medial part of the sclerotome migrate towards the notochord, surround it and later form vertebral bodies and intervertebral discs. The more laterally situated sclerotome cells participate in the formation of the neural arches, pedicles and ribs (Verbout, 1985; Christ and Wilting, 1992). Changes in the extracellular matrix are thought to be involved in the early steps of sclerotome formation by the synthesis of hyaluronic acid in the disrupting ventral epithelium (Solursh *et al.*, 1979). Most extracellular differentiation products characteristic of chondrogenic development only appear much later in the sclerotome, like CSPG-6 around day five.

The dermomyotome is the source of dermal connective tissue and skeletal muscle. First, it generates the myotome by proliferation and by elongation of cells at its cranial lip. The myotome represents a second layer underneath the dermomyotome/dermatome and consists of longitudinally arranged cells (Fig. 10). Myotome cells are first detectable in the cranio-medial corner of the somite and gradually extend caudally. This process continues in a ventro-lateral direction (Kaehn *et al.*, 1988). At this time, myotome cells are post-mitotic and express muscle-specific proteins such as desmin and early skeletal myosin. Before this histological and biochemical differentiation occurs, the precursor compartment of the myotome can already be distinguished by the presence of mRNA for myogenic basic helix-loop-helix transcription factors in the dorso-medial quadrant (Ott *et al.*, 1991; Pownall and Emerson, 1992). Interestingly, the dorso-lateral quadrant does not express myoD, although it will also give rise to skeletal muscle (hypaxial and limb muscle). Moreover, the population of cells expressing myoD at later stages lies in the dorso-medial lip of the dermomyotome and is therefore not the population of immediate myotome precursor cells, since myotome cells arise at its cranial lip (Kaehn *et al.*, 1988; Christ and Ordahl, 1995).

The differentiation of the cells located in the dorso-lateral quadrant is preceded by cell migration and proliferation. The premitotic myogenic precursor cells for the limb musculature leave the lateral margin of the dermomyotome, cross the Wolffian duct and the intermediate mesoderm and invade the limb bud mesoderm in a proximo-distal direction (Grim, 1970; Christ *et al.*, 1974, 1979; Chevallier *et al.*, 1977; Jacob *et al.*, 1978, 1979; Brand *et al.*, 1985). The epithelio-mesenchymal transition of the lateral edges of the dermomyotomes at limb bud levels has been shown to be mediated by the c-*met* receptor tyrosine kinase which is expressed in the dermomyotome (Bladt *et al.*, 1995). At the time of migration, myogenic cells express *Pax-3* (Bober *et al.*, 1994a; Williams and Ordahl, 1994). Within the limb bud, myogenic cells follow a proximo-distal gradient of "juvenility" (Brand, 1987; Brand-Saberi *et al.*, 1989). An involvement of *Pax-3* in myogenic cell invasion is supported by the fact that the murine *Pax-3*-mutant *splotch* does not possess myogenic cells in the limb bud, although these cells seem to have been generated by the dermomyotome (Franz *et al.*, 1993; Bober *et al.*, 1994a). This observation makes it likely that *Pax-3* somehow regulates the expression of molecules involved in cell migration, such as cell adhesion molecules, and only shortly before histological and biochemical differentiation myoD is expressed by the muscle precursor cells after they have entered the postmitotic phase.

Also at the cervical and flank levels, the dorsolateral quadrant of the somites gives rise to skeletal muscle. Epithelial buds from the dermomyotome together with the underlying myotome migrate ventrally into the abdominal wall (Fischel, 1895; Christ *et al.*, 1983, 1986). Finally, dermal fibroblasts leave the dermomyotome dorsally as individual cells, so that the myotome remains to mark the original segment boundaries.

Local interactions

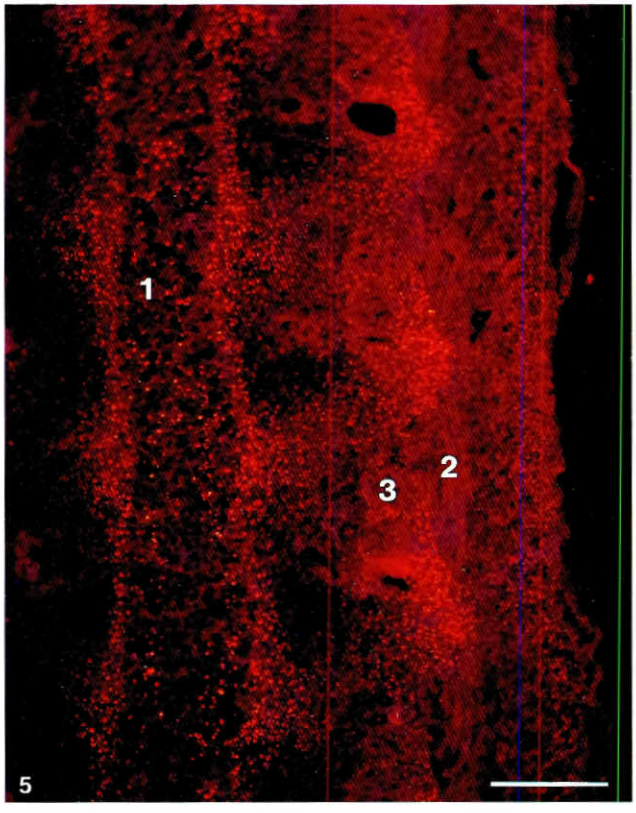
In contrast to somitogenesis which is an inherent process of the paraxial mesoderm, somite differentiation depends on local signalling between neighbouring structures (Avery *et al.*, 1956; Ellison *et al.*, 1969a,b; O'Hare, 1972a,b,c; Packard and Jacobson, 1976; Hall, 1977; Kenny-Mobbs and Thorogood, 1987; Christ *et al.*, 1992). Early observations concerning the significance of interactions between the axial structures and the somites go back to Holtzer (1952), Watterson and coworkers (1954) and Strudel (1955). Further experiments were carried out by Christ and coworkers (Christ *et al.*, 1972; Jacob *et al.*, 1974). However, first analyses at the molecular level have become possible in recent years. Most of the interactions described in this chapter have been established by microsurgical manipulations

Fig. 5. Anti chondroitin-6 sulphate-proteoglycan staining. *Frontal section of a 5 day chick embryo. Reactivity is stronger in the caudal sclerotome. Courtesy of H.H. Epperlein. 1 notochord, 2 myotome, 3 spinal nerve. Bar, 50 µm.*

Fig. 6. Anti-tenascin staining of a 5 day chick embryo. *Frontal section showing tenascin-positive reaction in the caudal sclerotome. Courtesy of H.H. Epperlein. 1 notochord, 2 myotome. Bar, 50 µm.*

Fig. 7. Stages of somite differentiation in the avian embryo. *Diagram showing putative regulatory genes expressed during somite formation and differentiation into dermomyotome and sclerotome. 1 neural tube, 2 notochord, 3 unsegmented paraxial mesoderm, 4 epithelial somite, 5 dermomyotome, 6 myotome, 7 sclerotome, 8 myogenic cells.*

Fig. 8. Whole-mount-*in situ* hybridization with a quail *Pax-1* probe in a stage 10 HH quail embryo. *Ventral aspect. Pax-1 starts to be expressed in the third somite cranial to the unsegmented paraxial mesoderm (arrow). Specimen provided by Thomas S. Mueller. Bar, 100 µm.*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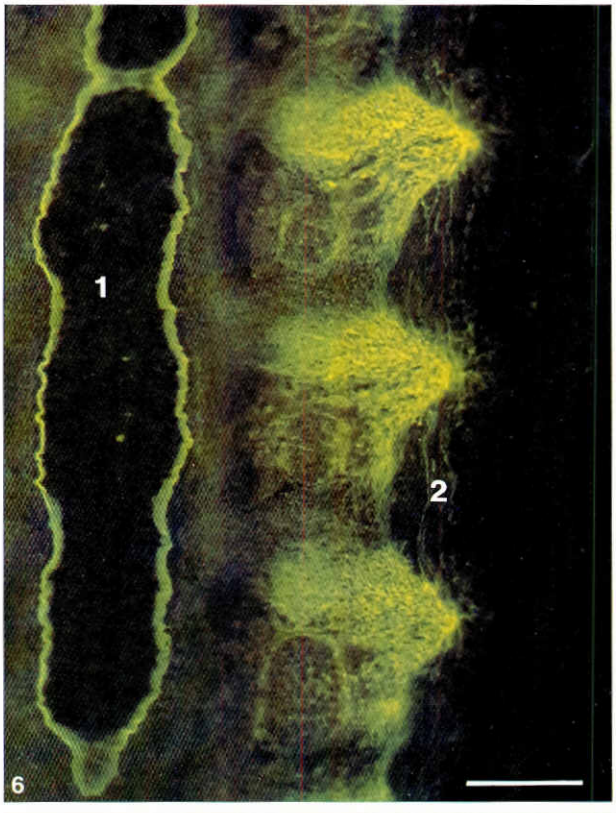
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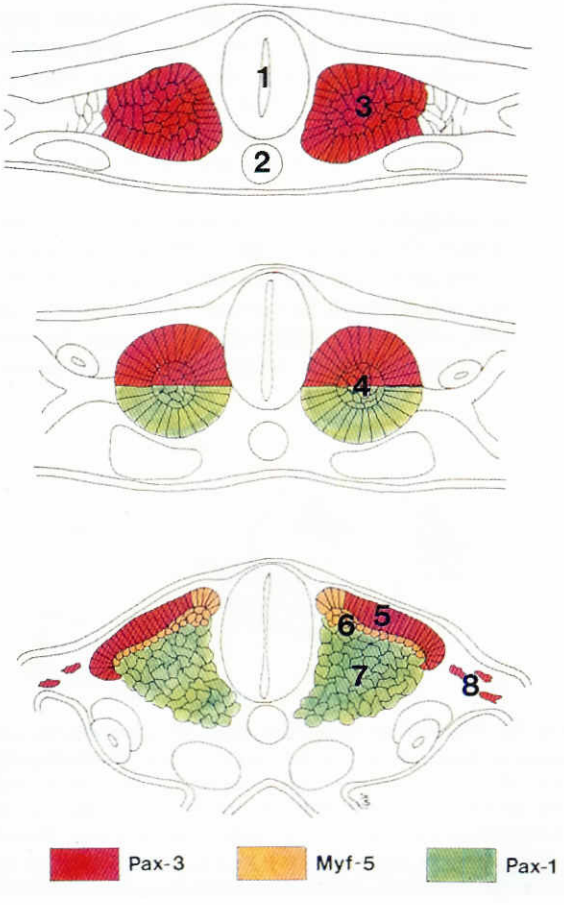
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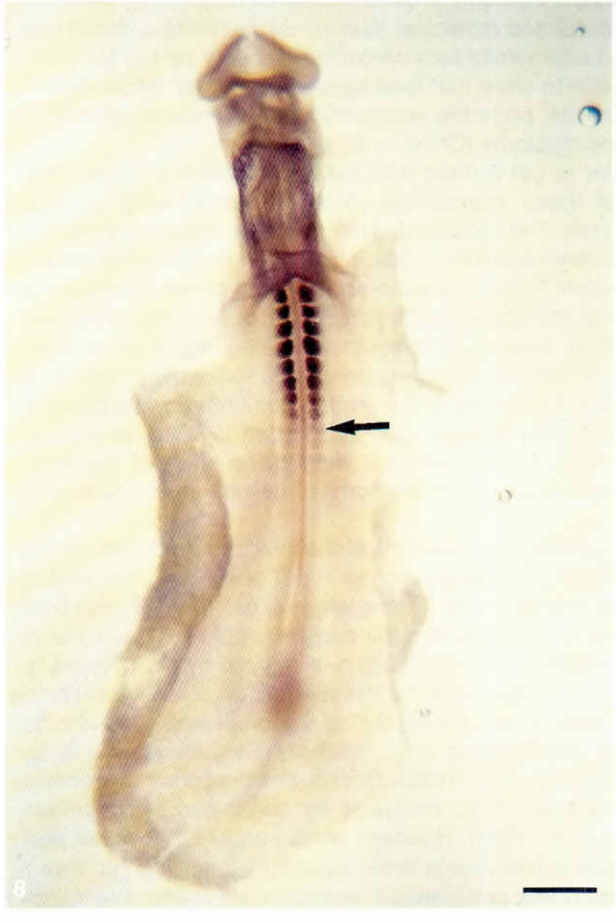


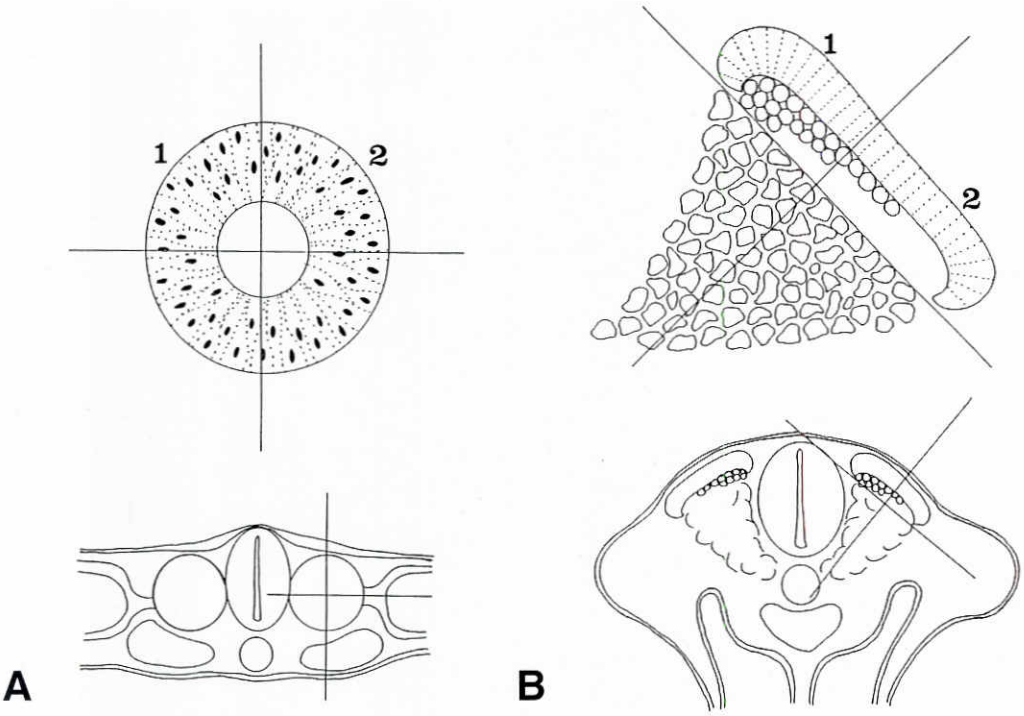


Fig. 9. During development, the somite undergoes rotation as a result of the lateral folding process of the embryo. *The dorso-medial quarter (1) of the somite comes to lie more dorsally and the dorso-lateral quarter (2) comes to lie more ventrally. A. Situation in the two-day embryo, B. Situation in the 3-day embryo.*

such as removal of structures and isotopic interspecific grafting or heterotopic insertion of extra-structures followed by immunohistochemical and molecular studies. By replacing a dorsal half of an epithelial somite by a ventral half (double ventral somites), it is possible to show that local signals control the differentiation of the somites, since the ventral half yields a normal dermomyotome and myotome (Christ *et al.*, 1992).

In order to get a more detailed insight into the source and nature of these interactions, different experimental set-ups were chosen: The ectopic implantation of an extra-notochord of a quail embryo laterally or medially to the unsegmented paraxial mesoderm leads to a suppression of myotome formation at the level of the operation (Brand-Saberi *et al.*, 1993; Pourquié *et al.*, 1993). Instead of a myotomal epithelium, a loose mesenchyme is formed. Moreover, the *Pax-1* expressing domain is extended in the vicinity of the grafted notochord, while desmin-immunoreactivity is negative (Figs. 11 and 12). This phenomenon has been interpreted as a ventralizing effect of the notochord, which it exerts on the somites as well as on the neural tube.

After experimental removal of the notochord prior to neural tube patterning, the acquisition of *Pax-1* positivity in the ventral part of the somite is lost (Ebensperger *et al.*, 1995). These findings led us to conclude that the expression of *Pax-1* as a mediator of vertebral column formation is induced by the notochord. This function is taken over by the ventral part of the neural tube (Brand-Saberi *et al.*, 1993), during the process of neural tube patterning (van Straaten *et al.*, 1988; van Straaten and Hekking, 1991; Goulding *et al.*, 1993). Recent observations have shown that the interaction is mediated by sonic hedgehog protein (Johnson *et al.*, 1994). However, sonic hedgehog does not prevent muscle differentiation in the somites, suggesting that *Pax-1* expression is only one aspect of notochord signalling. The inductive interaction between notochord and *Pax-1* expression in avian embryos is closely paralleled by defects found in the vertebrae of the murine *Pax-1*-mutant, *undulated* (Balling *et al.*, 1988). The interplay of notochord, *Pax-1*, and sclerotome (vertebral column) development is also seen in a murine notochord defect mutant, *Danforth's short tail* (*Sd*). In this mutant, the notochord degenerates prematurely which results in severe malformations, mainly in the lumbar and sacral region (Koseki *et al.*, 1993).

After extirpation of the neural tube, myotome differentiation is suppressed (Christ *et al.*, 1992). Although a second epithelial layer forms under the dorsally fused unpaired dermomyotome, the cells are reduced in number. They are not longitudinally arranged, and desmin expression is vestigial. However, the initiation of myogenesis is independent of the presence of the

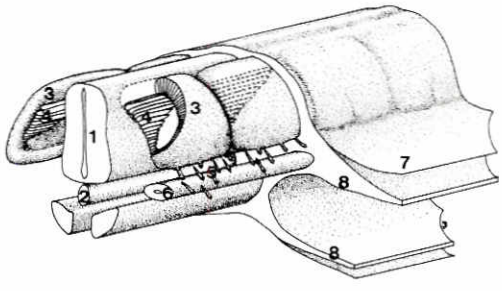
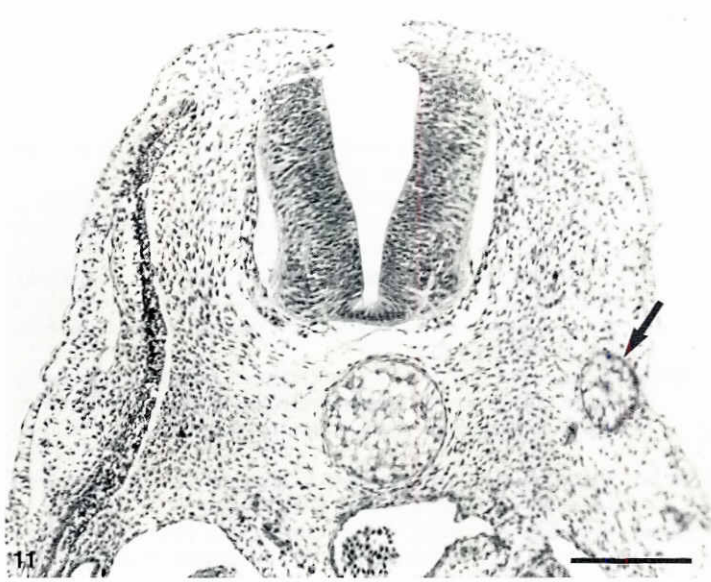


Fig. 10. Diagram showing the stage of myotome formation in the avian embryo. *In the first somite on the right the dermomyotome (3) is partially removed to show myotome cells in the cranio-medial corner of the somite. Myogenic cells (5) migrating from the ventrolateral dermomyotome towards the limb buds. 1 neural tube, 2 notochord, 3 dermomyotome, 4 myotome, 5 myogenic cells, 6 Wolffian duct, 7 ectoderm (partially removed), 8 lateral plate mesoderm (partially removed).*



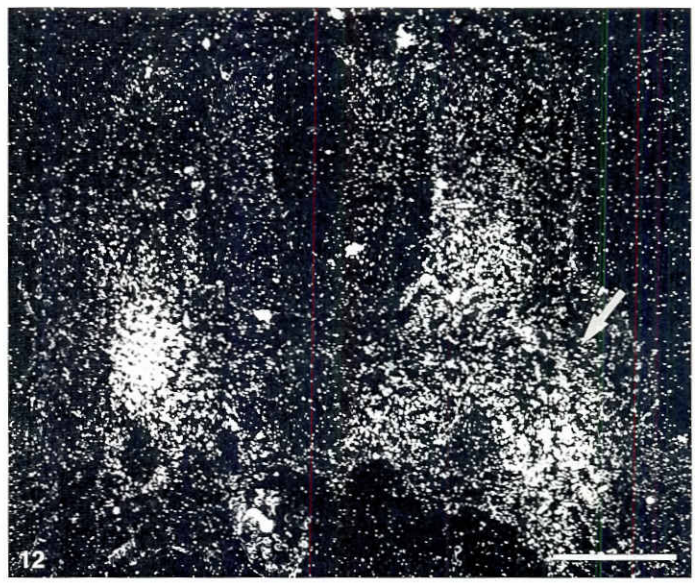


Fig. 11. Anti-desmin staining of a chick embryo with additional notochord (arrow) after 2 days of reincubation. *The dermomyotome is missing at the operated side. Bar, 50 µm.*

Fig. 12. *Pax-1* expression two days after grafting an additional notochord (arrow) laterally to the unsegmented paraxial mesoderm. *The transverse section shows expression of Pax-1 in the sclerotomes on both sides, but the expression at the operated side is extended compared to the control side. Dark-field. Bar, 50 µm.*

neural tube and even occurs in the neighbourhood of a grafted notochord. Transcripts of the avian homolog of myoD, CMDI, have been found in neural tube-extirpated embryos. In recipients of extra-notochords, transcripts are detectable in the myotome-like areas or in desmin-negative rudiments (Bober *et al.*, 1994a).

While we have been able to get insight into the control of epaxial muscle formation, the factors involved in the differentiation of muscle in the hypaxial domain are still largely unknown. Since these muscle groups differentiate normally in the absence of axial organs, their development is assumed to be either controlled by different mechanisms or to take place autonomously. In this respect, we have found that two closely related genes, *follistatin* and *flik* (*follistatin*-like gene) are differentially expressed in the dorsal compartments of the somites and are controlled by different signals.

If local interactions are involved in analogy to epaxial muscle, controlling signals might emanate from neighbouring structures

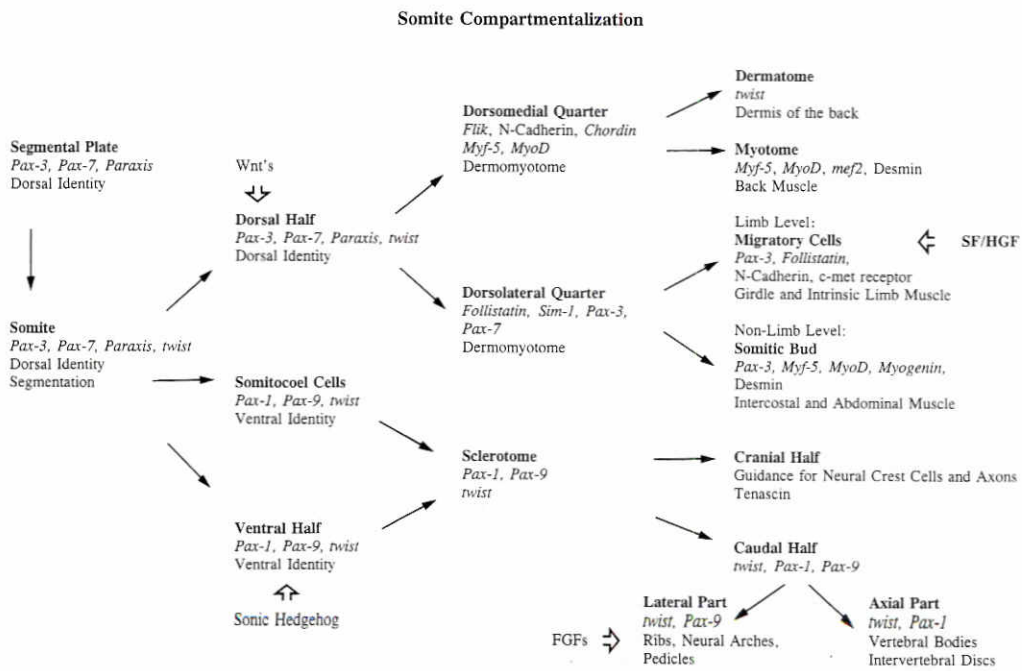


Fig. 13. Schematic diagram summarizing the process of somite compartmentalization and signalling.

such as the ectoderm, the intermediate mesoderm, the Wolffian duct or the lateral plate mesoderm. Evidence for such an influence on the lateral half of the paraxial mesoderm has recently been given by Pourquié *et al.* (1995) as well as by *in vitro* experiments (Gamel *et al.*, 1995).

Regarding the limb muscles, evidence exists that at least their patterning which involves migration and local arrangement into muscle blastemata is controlled by the limb mesoderm.

The myoblasts encounter various substrates, during their directed migration to and within the limb buds, such substrates include the ECM surrounding the somites and the intermediate mesoderm and Wolffian duct, and the cells and ECM within the limb buds. The muscle precursor cells respond to signals on their migration routes and follow them in a distal direction (Brand *et al.*, 1985; Brand-Saberi *et al.*, 1989). Fibronectin is necessary for this process, since migration ceases when antibodies against the main fibronectin binding site are applied (Brand-Saberi *et al.*, 1993). However, directionality is believed to be inferred from the distribution not of fibronectin, but of hyaluronic acid (Krenn *et al.*, 1991; Brand-Saberi and Krenn, 1991) and cellular components, possibly cell adhesion molecules. Recently, N-cadherin was demonstrated to be present on myogenic cells and on the cells of the distal dorsal and ventral limb bud mesoderm (Brand-Saberi *et al.*, 1995). Differentiation of limb muscle cells does not depend on a completed migration within the limb bud, but correct patterning of the musculature needs signals from the lateral plate mesoderm (Jacob *et al.*, 1982; Brand-Saberi and Krenn, 1991).

To fully understand some of these complex interactions between early embryonic structures at the morphogenetic, transcriptional and differentiation level has become one of the most challenging tasks in developmental biology. The establishment of the primitive body plan is a rewarding model system to study such interactions combining traditional and modern techniques. We have given here an overview on topical concepts concerning the prerequisites and the consequences of somite compartment formation. The isolation of further genes that show a compartment-specific expression promises new insights into the control of somite differentiation.

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