Anat Embryol (2000) 202:369–374

ORIGINAL ARTICLE

Ruijin Huang · Bodo Christ

Origin of the epaxial and hypaxial myotome in avian embryos

Accepted: 27 June 2000

Abstract The myotome originates from the dermomyotome. Controversy surrounds the location of myotome precursor cells within the dermomyotome and their segregation from the dermomyotome. Here we addressed the problem of myotome formation by labeling dermomyotome cells using the quail-chick marking technique. We carried out five series of transplantation and replaced: (1) the medial third, (2) the intermediate third, (3) the lateral third, (4) the cranial half, (5) the caudal half of a thoracic dermomyotome. The grafting procedures were performed in HH-stages 15–17 of quail and chick embryos. The chimeras were reincubated for 2 days up to HH-stages 24–25. All of the grafted parts contributed to the myotome. The epaxial myotome is derived from the medial third of the dermomyotome, while the hypaxial myotome is formed by both the intermediate and lateral third of the dermomyotome. Ep- and hypaxial myotome domains meet in the thickest part of the myotome that is situated in the middle of its ventrolateral axis. Myotome growth in the epaxial domain begins earlier than in the hypaxial domain. Cranial and caudal edges of the dermomyotome contribute equally to both the epaxial and hypaxial myotomes. The first born myotome cells are located in the lateral part of the epaxial myotome and development then proceedes in medial and lateral directions.

Key words Somite · Dermomyotome · Epaxial myotome · Hypaxial myotome · Quail-chick marking technique

Introduction

Somites form on either side of the neural tube by segmentation of the paraxial mesoderm. They are epithelial spheres enclosing a cluster of mesenchymal cells, the somitocoel cells (reviewed Christ and Ordahl 1995). In response to signals from adjacent structures, the ventral somite cells lose their epithelial organization to form the mesenchymal sclerotome that gives rise to the axial skeleton and the ribs. The mesenchymal somitocoel cells contribute to the sclerotome (Huang et al. 1996). The dorsal part of the somite retains its epithelial organization and is known as the dermomyotome because it contains precursors for both muscle and dermis (reviewed Christ and Ordahl 1995). The dermomyotome is an epithelial layer whose four edges or lips (medial, lateral, cranial, caudal) are curved inwards. It was first described by Fischel (1895) as the source of myogenic progenitor cells. Recent experimental studies using the chick-quail marking technique confirmed this hypothesis and additionally supplied evidence that the dermomyotome gives rise to precursors for all of the epaxial (back) and hypaxial (limb and ventral body wall) muscle (Christ et al. 1977, 1978). Epaxial muscle was suggested to originate from the medial half and hypaxial muscle from the lateral half of the somite (Ordahl and Le Douarin 1992).

The myotome represents a layer of spindle-shaped and longitudinally oriented postmitotic embryonic myoblasts and is located beneath the dermomyotome, which consists of radially oriented columnar cells. Whereas the epaxial myotome has a homogenous fate in that it forms homogeneously the back muscles of all trunk regions, the hypaxial muscles develop heterogeneously depending on the axial level under consideration. At limb levels and in the occipital region the lateral part of the dermomyotome de-epithelializes and sends myogenic cells into the limb somatopleure and into the floor of the branchial arches, where the cells differentiate into limb and tongue muscle (reviewed Christ and Ordahl 1995). At interlimb level the lateral myotome is formed by mechanisms similar to those of the medial myotome formation (Cinnamon et al. 1999; Denetclaw and Ordahl, 2000). However, the location of muscle precursors within the dermomyotome and the mode of their translocation into the myotome have been described controversially. Williams (1910) first proposed myotome cells to be formed from the medial edge of the dermomyotome, where early myogenic determination

R. Huang, B. Christ (✉)
Institute of Anatomy, University of Freiburg, P.O. Box 111
79001 Freiburg, Germany
e-mail:christb@uni-freiburg.de
Tel.: +49-761-203-5088, Fax: +49-761-203-5091

genes are expressed (reviewed Borycki and Emerson 2000). Kaehn et al. (1988) proposed that the early myotome cells emerge from the mediocranial corner of the dermomyotome. Using fluorescent vital dye labeling of dermomyotome cells, Denetclaw et al. (1997) and Denetclaw and Ordahl (2000) suggested that muscle precursor cells for the epaxial and hypaxial myotomes are predominantly located in the dorsomedial and ventrolateral dermomyotome lips, respectively. In contrast, by means of combinations of DiI labeling, proliferation assay and micromanipulation Kahane et al. (1998a, b) described the myotome to be formed by two waves of cells from the dermomyotome. The first population of "pioneer cells" was described to originate along the dorsomedial edge. Thereafter a second cell population was suggested to be derived from both the cranial and caudal edges of the dermomyotome but not from the dorsomedial and the ventrolateral edge. Due to the limitation of the fluorescent vital dye labeling technique, only the early but not the late myotome development could be followed in these studies.

In this study we investigated the formation of epaxial and hypaxial myotomes in late stages using the quail-chick cell marking technique (Le Douarin 1969). We carried out five series of experiments: transplantation of (1) the medial third, (2) the intermediate third, (3) the lateral third, (4) the cranial half, (5) the caudal half of the dermomyotome. Thus, these regions of the dermomyotome could be followed with respect to their participation in myotome formation. Our results show that the epaxial myotome is formed by the medial third of the dermomyotome, while the hypaxial myotome develops from the lateral two thirds. The cranial and caudal dermomyotome halves contribute equally to the developing myotome.

Materials and methods

Preparation of chick and quail embryos Fertilized chicken and quail eggs were obtained from a local hatchery. The eggs were incubated at 37.8°C in a humidified (75%) incubator for 2 days. Experiments were performed on embryos at stages 15 to 17 (according to Hamburger and Hamilton 1951). Chimeras were reincubated for two days and then processed for whole-mount in situ hybridization and antibody staining.

Grafting procedures To clarify the contribution of different parts of the dermomyotome to the myotome, five grafting series were performed. We grafted (1) the medial third, (2) intermediate third, (3) lateral third, (4) cranial half and (5) caudal half of the dermomyotome of a thoracic somite (somite stages IV–X, according to Christ and Ordahl 1995) homotopically from quail to chick. The operations were usually located at somite levels 21–25 of HH-stages 15–17 embryos. A donor embryo (quail) was first isolated from the yolk, spread out over a plastic culture dish with a 2-mm-thick layer of 2% agarose and fixed in place with needles. One part of a single dermomyotome (medial, intermediate and lateral thirds, cranial and caudal halves) with attached surface ectoderm was isolated with a tungsten needle and stained using a blunt glass needle coated with 2.5% agar containing 2% nile blue. The surface ectoderm allowed orientation of the graft.

Manipulation on the host embryo (chick) was performed in ovo. After windowing the eggshell and the subjacent shell membrane, a small hole in the vitelline membrane covering the prospective surgery region was made with a tungsten needle. One part of an individual dermomyotome (medial, intermediate and lateral thirds, cranial and caudal halves) with its surface ectoderm was removed with a tungsten needle and a mouth-operated micropipette. Finally, the quail dermomyotome part was transferred to the chick surgery site with a Spemann pipette and fixed in the corresponding site where the chick dermomyotome part had been already removed. After the grafting procedure chimeras were reincubated for 2 days up to HH-stages 24–25.

Whole-mount in situ hybridization. All chick embryos were washed in PBS and then fixed overnight in 4% paraformaldehyde at 4°C. Anti-sense RNA probes of *MyoD* (a 1518 bp probe was kindly provided by Dr. Bruce Paterson) were labelled with digoxigenin and whole-mount in situ hybridization was performed as described by Nieto et al. (1996). The alkaline phosphatase conjugated digoxigenin was detected with fast red colour reaction (Boehringer, Mannheim, Germany).

Immunohistochemistry After the whole-mount in situ hybridization the alkaline phosphatase was inactivated by acetic acid (Merck) and refixed in 4% paraformaldehyde. Embryos were incubated for 4 h in 10% horse serum (in PBT), incubated overnight with an anti-quail antibody (QCPN; Developmental Studies Hybridoma Bank, Iowa City, Iowa, 1:100 in 10% horse serum/PBT), washed in PBT, incubated overnight in secondary antibody (alkaline phosphatase-conjugated goat anti-mouse IgG antibody, DAKO 1:1000, in 10% horse serum/PBT), washed in PBT, then in alkaline phosphatase buffer. Quail nuclei reveal a blue colour after colour reaction with nitroblue tetrazolium (NBT) and X-phosphate (BCIP; Boehringer). Embryos were photographed using a dissection microscope fitted with a camera. Then embryos were cryoembedded and serially sectioned at 30 µm in transversal planes using a cryostat. To better demonstrate the dermomyotome, sections were stained with an anti-desmin monoclonal antibody (DAKO, 1:400, in 10% horse serum/PBT). Peroxidase-conjugated goat anti-mouse antibody (Sigma, 1:300, in horse serum/PBT) was used as secondary antibody and diaminobenzidine (DAB) as chromogen (brown reaction product).

Some chimeras were prepared for paraffin sectioning. They were fixed in Serra's solution (Serra 1946) overnight. After dehydration, the embryos were embedded in paraffin and sectioned serially at 8 µm in transversal planes. Double labeling with an anti-quail antibody and a polyclonal anti-desmin antibody was performed as described previously (Huang et al. 2000).

Results

Transplantation of different parts of a dermomyotome from quail to chick embryos was used to analyze epaxial and hypaxial myotome formation in thoracic level somites (somites 21–25) of HH-stage 15–17 embryos. After 2 days of reincubation, the development of epaxial and hypaxial myotome cells was then followed up by whole-mount in situ hybridization with *MyoD* probe and immunohistochemical staining with an anti-quail and an anti-desmin antibody. A total of 60 transplantations were performed and 30 embryos could be analyzed for this study (at least 3–5 embryos for each experimental series). The other 30 embryos died before fixation or showed malformations, or the graft was disappeared.

The medial third of the dermomyotome forms the epaxial myotome

We grafted the medial third of the dermomyotome at HH-stages 15–17 (Fig. 1A). A typical example of this grafting series is shown in Fig. 1B,C. The experiment was performed with somite 24 of a HH-stage 15 em-

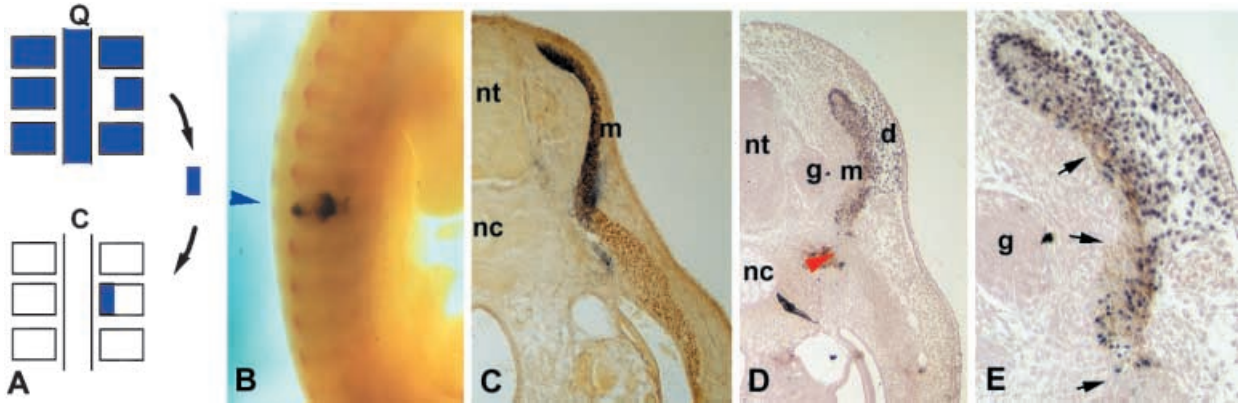


Fig. 1A–E Grafting of the medial third of the dermomyotome. **A** Diagram of grafting procedure (*Q* quail chick, *C* chick). **B** Whole-mount in situ hybridization with *MyoD* probe (red) and labeling with anti-quail antibody (QCPN; blue, *arrowhead*) after grafting procedure at HH-stage 15 and a reincubation period of 2 days. *MyoD* expression (red) shows the extension of the myotomes. **C** Cross section of the embryo in **B** at the level of the operated somite. Section stained additionally with QCPN (blue) and anti-desmin antibody to label myotome cells (brown). **D** Cross section of a chimera after grafting at HH-stage 17 and 2 days of reincubation (*red arrowhead* boundary between epaxial and hypaxial myotomes). **E** Higher magnification of the section in **D** (*arrows* myotome cells of chicken origin, *d* dermis, *g* dorsal root ganglion, *m* myotome, *nc* notochord, *nt* neural tube)

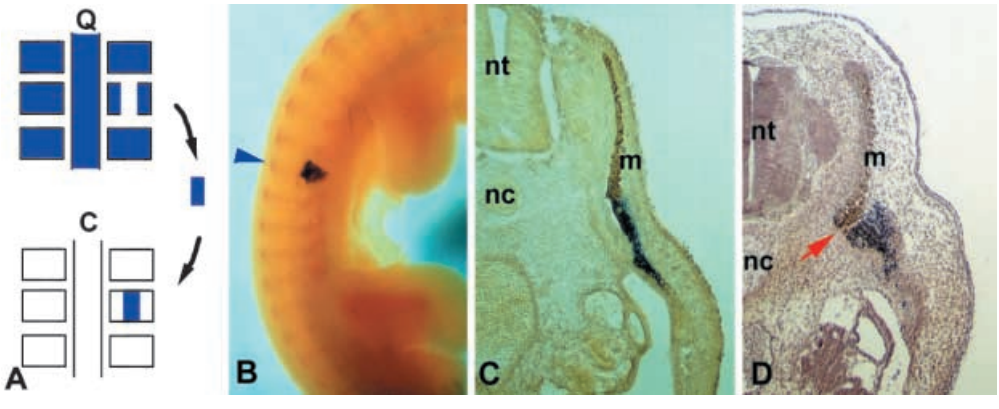
bryo. The *MyoD* expression shows a normal pattern of the myotome. The operated somite is located at the middle trunk level. The quail-derived myotome cells have expanded into a ventro-lateral direction and occupy the medial myotome domain (Fig. 1B). The cross section shows desmin antibody-labeled myotome cells (Fig. 1C). At notochord level (about the middle of the entire myotome layer), the myotome grows inwards and shows a triangular shape. This part has been regarded as the boundary between epaxial and hypaxial myotome domains (Denetclaw and Ordahl 2000). Quail cells are located in the epaxial part of the myotome. Quail-derived myotome cells extend laterally to the triangular part of the myotome, showing that the epaxial myotome cells are derived from the medial third of the dermomyotome. It is very interesting to notice that the quail-derived myotome cells did not extend to the border between epaxial and hypaxial myotome when the transplantation was carried out in HH-stage 17 (Fig. 1D). In this case, some myotome cells of the epaxial domain are of chick origin (Fig. 1E).

The lateral two-thirds of the dermomyotome form the hypaxial myotome

We grafted intermediate and lateral thirds of the dermomyotome at HH-stages 15–17 (Figs. 2,3). Quail cells from the intermediate third of the dermomyotome are located in the myotome laterally to the border between epaxial and hypaxial domains (Fig. 2B,C). There is a very sharp boundary in the triangular part of the myotome between chick cells in the epaxial domain and quail cells in the hypaxial domain (Fig. 2D). Quail cells could never be found in the lateralmost part of the myotome.

After transplantation of the lateral third of the dermomyotome, quail-derived myotome cells extend laterally into the ventro-lateral lip of the myotome (Fig. 3). Medially, quail-derived cells stay at some distance from the border between epaxial and hypaxial, whereas myotome cells from the intermediate third of the dermomyotome extend

Fig. 2A–D Grafting of the intermediate third of the dermomyotome. **A** Diagram of grafting procedure. **B** Whole-mount in situ hybridization with *MyoD* probe (red) and labeling with anti-quail antibody (QCPN; blue, *arrowhead*) after a reincubation period of 2 days. **C** Cross section of the embryo in **B**. Section stained with QCPN and anti-desmin antibody to label myotome cells (brown). **D** Paraffin section. Note the very sharp boundary of the quail-derived myotome cells in the triangular part of the myotome (*red arrow*)



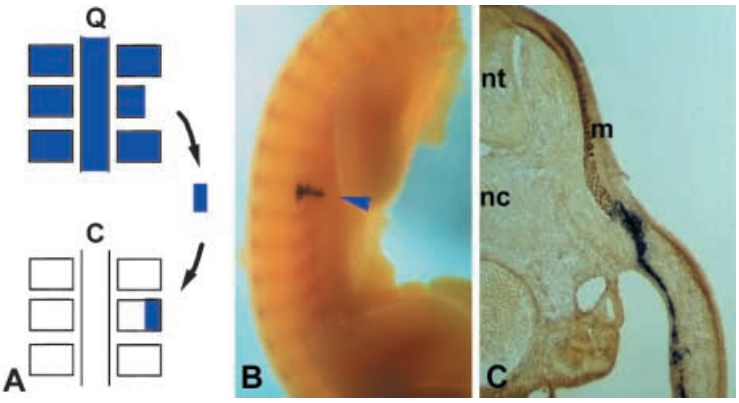


Fig. 3A–C Grafting of the lateral third of the dermomyotome. **A** Diagram of grafting procedure. **B** Whole-mount in situ hybridization with *MyoD* probe (red) and labeling with anti-quail antibody (QCPN; *arrowhead*, blue) after a reincubation period of 2 days. MyoD expression (red colour) shows the segmental pattern and the extension of myotomes. **C** Cross section of the embryo in **B**. Myotome cells are stained with QCPN (blue) and anti-desmin antibody (brown)

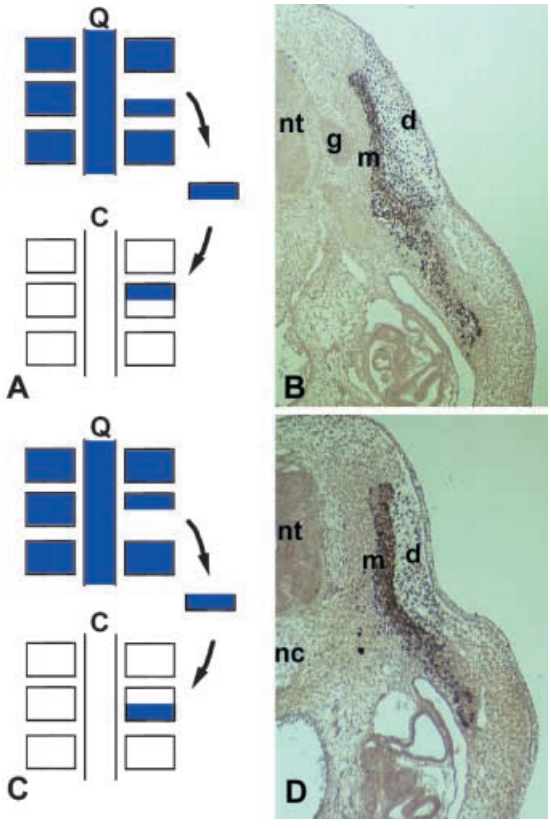


Fig. 4A–D Grafting of cranial and caudal halves of the dermomyotome at thoracic level. **A, C** Diagrams of grafting procedures. Experiments are carried out at HH-stages 15–16. The embryos were reincubated for 2 days. **B, D** Cross sections of the operated regions stained with anti-desmin antibody to label myotome cells (brown) and with anti-quail antibody (QCPN) to label quail cell nuclei. The results show that both the cranial (**A, B**) and the caudal (**C, D**) halves of the dermomyotome contribute equally to myotome formation

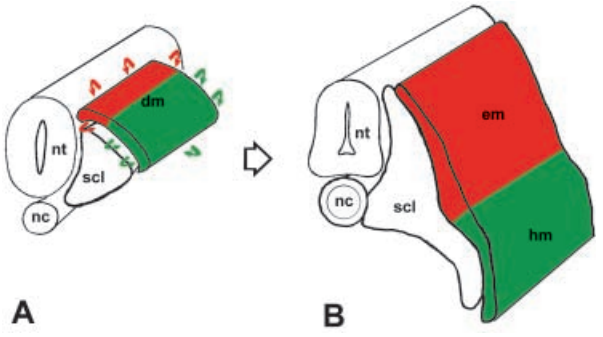


Fig. 5A,B Summary of epaxial and hypaxial myotome formation in thoracic somites. **A** A thoracic somite (at somite levels 21–25, somite-stages IV–X, according to Christ and Ordahl 1995) of a 2-day embryo. Dermomyotome (*dm*; red and green part) and sclerotome (*scl*). The red part represents the medial third of the dermomyotome; the green part its lateral two thirds, *arrows* mark the detachments of myotome precursors from the dermomyotome. **B** Somite at thoracic level of a 4-day old chick embryo. The red part represents the epaxial myotome and the green part the hypaxial myotome. The medial third of the dermomyotome gives rise to the epaxial myotome, its lateral two-thirds forms the hypaxial myotome (*dm* dermomyotome, *em* epaxial myotome, *hm* hypaxial myotome, *scl* sclerotome)

medially up to the border between both myotome domains (s.a.). The lateral part of the myotome that is derived from the intermediate third of the dermomyotome overlaps with the medial part of the myotome that has originated from the lateral third of the dermomyotome. Therefore, myotome cells from the intermediate and lateral thirds of the dermomyotome form together the hypaxial myotome.

The cranial and caudal dermomyotome halves contribute equally to the myotome

To investigate the contribution of the cranial and caudal dermomyotome halves to the myotome we grafted these parts and reincubated the chimeras for 2 days (Fig. 4). A typical embryo for the cranial dermomyotome-half grafting is shown in Fig. 4B. This cross section is situated in the caudal part of the cranial somite half. The dorsal root ganglion is cut through its caudal end. Quail cells contribute to the entire myotome layer and can be found also in the back dermis. It is very interesting to notice that the ventrolateral edge of quail-derived dermis corresponds to the boundary between epaxial and hypaxial parts of the myotome. Quail cells from the caudal dermomyotome half contribute also to the entire myotome layer and to the dermis of the epaxial domain (Fig. 4D). This is shown in a cross section from the cranial part of the caudal somite half. These results show that the cranial and caudal dermomyotome halves contribute equally to the myotome.

Discussion

In this paper we have studied the contribution of different parts of the dermomyotome to the formation of the myotome. We subdivided the dermomyotome into a medial third, intermediate third, lateral third, cranial half and caudal half and grafted these pieces from quail to chick. In this way we were able to follow the progeny of the grafted cells within the developing myotome. Our results show that the cells of the epaxial myotome arise from the medial third of the dermomyotome, while the hypaxial myotome cells originate from the lateral two thirds. The cranial and caudal halves of the dermomyotome contribute to both the epaxial and hypaxial domains.

Cell tracing experiments by Christ et al. (1978), Denetclaw et al. (1997), Denetclaw and Ordahl (2000), Kahane et al. (1998a, b), and Cinnamon et al. (1999) have supplied evidence that the epaxial and hypaxial myotomes originate exclusively from the dermomyotome and not by integration of sclerotome cells as was postulated by Mestres and Hinrichsen (1976). The questions of how the cells leave the dermomyotome and in which ways they enter the myotome are not quite clear. Fischel (1895), Rabl (1888), Williams (1910) and Ede and El-Gadi (1986) concluded that the myotome cells originate mainly from the dorsomedial lip of the dermomyotome. Langman and Nelson (1968), on the other hand, favoured the idea that the myotome cells do not originate from the dorso-medial lip, but rather throughout the whole length of the overlying dermomyotome by delamination. This possibility, however, has not been supported by cell tracing experiments. After vital dye labeling studies Denetclaw et al. (1997) and Denetclaw and Ordahl (2000) have shown that the early myotome-forming cells are mainly situated in the dorsomedial and ventrolateral dermomyotome lips. These results are in contrast to the suggestions by Kaehn et al. (1988) who studied early myotome formation by use of a polyclonal anti-desmin antibody and by histochemical demonstration of acetylcholine esterase activity and concluded that the birth of epaxial myotome cells mainly occurs along the cranial edge of the dermomyotome. Kahane et al. (1998a,b) proposed that myotome cells emerge in early stages from the dorsomedial edge as “pioneer fibers” forming a longitudinal scaffold that serves as a substrate for the addition of subsequent waves of myotome cells coming from the cranial and caudal dermomyotome edges. According to Kahane et al. (1998a,b) cells born in the dorsomedial lip first translocate along the medial lip, and then reach the cranial and caudal edges, from which they elongate into the myotome. A similar mechanism for the formation of the hypaxial myotome has been proposed by Cinnamon et al. (1999).

Our results support those of Kaehn et al. (1988) as well as those of both Ordahl’s and Kalcheim’s groups (Kalcheim et al. 1999, Ordahl et al. 2000). The process of epaxial and hypaxial myotome formation is similar but myotome growth expands in opposite directions; the epaxial myotome grows more rapidly than the hypaxial one (Fig. 5). The earliest born cells (“Kaehn cells” according to Denetclaw and Ordahl) are located in the middle of the myotome close to the border between the epaxial and hypaxial domains. These cells may belong to the pioneer fibers of Kahane et al. (1998) that escape early from the dorsomedial lip of the dermomyotome.

The assumption by Christ et al. (1978), that myotome cells originate from all four edges of the dermomyotome is confirmed by our results. It is interesting that the cranial and caudal edges provide cells to all parts of the myotome. The fact that the intermediate third of the dermomyotome gives rise to many myotome cells clearly shows that the dorsomedial and ventrolateral lips are not required for translocation of cells from the dermomyotome into the myotome. This is in line with the suggestions by Kahane et al. (1998a, b) and Cinnamon et al. (1999) that myotome cells become detached from the cranial and caudal edges of the dermomyotome. It furthermore explains the origin of epaxial myotome cells even after insufficiency of the dorsomedial dermomyotome lip after removal of the dorsal neural tube (R. Huang, G.M. Cann, F.E. Stockdale, B. Christ, unpublished work). Our data do not exclude the observation by Kahane et al. (1998a, b) and Cinnamon et al. (1999) that prospective myotome cells may leave their birth places in the dorsomedial and ventrolateral lips to reach the cranial and caudal edges from which they enter the myotome. This suggestion could explain the elongation and orientation and migration of the myotome cells along a scaffold of longitudinally oriented pioneers and does not require a rounding-up or a changing of orientation of these cells. The molecular control of the detachment of myotome precursors from the dermomyotomal edges and their migration within the myotome remains to be studied.

Acknowledgements We are grateful to Mrs. S. Antoni, Mr. G. Frank, Mrs. E. Gimbel, Mrs. L. Koschny and Mrs. M. Schüttoff for excellent technical assistance and to Mrs. U. Uhl for typing the manuscript. We thank Dr. Jörg Wilting for critical reading and his comments. This study was supported by a grant from the Deutsche Forschungsgemeinschaft (Ch 44/14-1).

References

Borycki AG, Emerson CP (2000) Multiple tissue interactions and signal transduction pathways control somite myogenesis. Curr Top Dev Biol 48:165–224

Christ B, Ordahl CP (1995) Early stages of chick somite development. Anat Embryol 191:381–396

Christ B, Jacob HJ, Jacob M (1977) Experimental analysis of the origin of the wing musculature in avian embryos. Anat Embryol 150:171–186

Christ B, Jacob HJ, Jacob M (1978) On the formation of the myotomes in avian embryos. An experimental and scanning electron microscopic study. Experientia: 34:514

Cinnamon Y, Kahane N, Kalcheim C (1999) Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome. Development 126:4305–4315

Denetclaw WF, Ordahl CP (2000) The growth of the dermomyotome and formation of early myotome lineages in thoracolumbar somites of chicken embryos. Development 127:893–905

Denetclaw WF, Christ B, Ordahl CP (1997) Location and growth of epaxial myotome precursor cells. Development 124:1601–1610

Ede DA, El-Gadi AO (1986) Genetic modifications of developmental acts in chick and mouse somite development. In: Bellairs R, Ede DA, Lash JW (eds) Somites in developing embryos. NATO ASI Series, vol 119, Plenum Press, New York London, pp 209–224

Fischel A (1895) Zur Entwicklung der ventralen Runpf- und Extremitätenmuskulatur der Vögel und Säugethiere. Morphol Jb 23:544–561

Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. J Morphol 88:49–92

Huang R, Zhi Q, Neubüser A, Müller TS, Brand-Saberi B, Christ B, Wilting J (1996) Function of somite and somitocoele cells in the formation of the vertebral motion segment in avian embryos. Acta Anat 155:231–241

Huang R, Zhi Q, Schmidt C., Wilting J, Brand-Saberi B, Christ B (2000) Sclerotomal origin of the ribs. Development 127: 527–532

Kaehn K, Jacob HJ, Christ B, Hinrichsen K, Poelmann RE (1988) The onset of myotome formation in the chick. Anat Embryol 177:191–201

Kahane N, Cinnamon Y, Kalcheim C (1998a) The cellular mechanism by which the dermomyotome contributes to the second wave of myotome development. Development 125:4259–4271

Kahane N, Cinnamon Y, Kalcheim C (1998b) The origin and fate of pioneer myotomal cells in the avian embryo. Mech Dev 74: 59–73

Kalcheim C, Cinnamon Y, Kahane N (1999) Myotome formation: a multistage process (Special issue on myogenesis) Cell Tissue Res 296: 161–173

Langman J, Nelson GR (1968) A radioautographic study of the development of the somite in the chick embryo. J Embryol Exp Morphol 19:217–226

Le Douarin N (1969) Particularités du noyau interphasique chez la caille japonaise (*Coturnix coturnix japonica*). Utilisation de ces particularités comme “marquage biologique” dans les recherches sur les interactions tissulaires et les migrations cellulaires au cours de l’ ontogenèse. Bull Biol Fr Belg 103:435–452

Mestres P, Hinrichsen K (1976) Zur Histogenese der Somiten beim Hühnchen. J Embryol Exp Morphol 36:669–693

Nieto MA, Patel K, Wilkinson DG (1996) In situ hybridization analysis of chick embryos in whole mount and tissue sections. Methods Cell Biol 51:219–235

Ordahl CP, Le Douarin NM (1992) Two myogenic lineages within the developing somite. Development 114:339–353

Ordahl CP, Williams BA, Denetclaw W (2000) Determination and morphogenesis in myogenic progenitor cells: an experimental embryological approach. Current Top Dev Biol 48: 319–367

Rabl C (1888) Über die Differenzierung des Mesoderms. Verh Anat Ges 2:140–146

Serra JA (1946) Histochemical tests for protein and amino acids: the characterization of basic proteins. Stain Technol 21:5–18

Williams LW (1910) The somites of the chick. Am J Anat 11: 55–100