Myotome meanderings

Cellular morphogenesis and the making of muscle

Georgina E. Hollway^{†+} & Peter D. Currie[†]

MRC Human Genetics Unit, Edinburgh, UK

The formation of muscles within the vertebrate embryo is a tightly orchestrated and complex undertaking. Beyond the initial specification of cells to become muscle are several complex cellular movements and migrations, which lead to the positioning of muscle precursors at specific locations within the embryo. The consequent differentiation, elongation and striation of these cells results in the formation of individual muscles. Investigation of the *in vivo* morphogenesis of individual vertebrate muscle cells has only recently begun, and is being approached through the use of sophisticated cell labelling and lineage analysis techniques. However, a consensus about the mechanisms involved has yet to be achieved. This review outlines vertebrate embryonic muscle formation in chick, fish and mice, focusing on the embryonic myotome, which generates both the axial musculature and the appendicular muscle of the fins and limbs. We highlight the points of consensus about, and the complexity of, this developmental system, and propose an evolutionary context for the basis of these understandings.

EMBO reports 4, 855–860 (2003)

doi:10.1038/sj.embor.embor920

Making the amniote myotome

In all vertebrates, the skeletal muscle of the body axis is chiefly derived from an early embryonic compartment, known as the myotome. Within amniote embryos, the myotome is formed from a transient epithelial structure called the dermomyotome, which itself forms from the somites. The molecular and cellular basis of the morphogenetic transition from dermomyotome to myotome has been a matter of intense debate. In amniote embryos, it has been established that the myotome forms as a thin sheet of muscle tissue that expands in each somite in a manner co-ordinated with the expansion of the overlying dermomyotomal epithelium.

Myotome precursor cells are known to translocate from the dermomyotome to form the underlying myotome layer. However, the mechanism whereby these myotomal precursors are generated and stimulated to migrate has remained contentious. Some pioneering studies suggested that the cells of the early myotome all originate from one specific position within the dermomyotome, the dorsal medial lip (DML), which is the most axial region of the dermomyotome and is juxtaposed to the dorsal aspect of the neural tube (Williams, 1910; Hamilton, 1952; Boyd, 1960). Cells from this region were believed to consequently migrate under the dermomyotomal epithelium ventrally and laterally to generate the myotome. Another early model of myotome formation, based on observations of somite formation in a variety of species, suggested that myotomal cells originate not only within the DML, but also along the entire ventrolateral extent of the dermomyotome (Remak, 1855; His, 1888; Bardeen, 1900). This second model was supported by a later study (Langman & Nelson, 1968) of cell proliferation in the chick somite, which identified mitotically active tritiated thymidine-labelled cells beneath the entire dermomyotome.

The salient features of the two contradictory models that have arisen from these and subsequent studies are summarized in Fig. 1. Kalcheim and colleagues have expanded on the original observations by adapting cell-labelling approaches, coupling birth-dating assays such as thymidine pulse chase experiments with the injection of the lineage tracking dye Dil to investigate the origins of the cells that give rise to the myotome and its subsequent growth (Kahane *et al.*, 1998a,b, 2001, 2002; Cinnamon *et al.*, 1999, 2001). As a result of their studies, they believe that the myotome arises from the dermomyotome through several waves of migration (Fig. 1A–C). The first wave of progenitors or pioneer cells (named after the muscle pioneer cells that are present in zebrafish embryos, see below) arises in the dorsomedial region of the newly formed epithelial somite, in line with other studies (Christ *et al.*, 1978; Ordahl & Le Douarin, 1992). Such an early medial–lateral polarization is consistent with gene expression profiles obtained from this developmental stage and with the early commitment of dorsomedial cells to the myogenic lineage. As the dermomyotome changes from its epithelial state, a group of postmitotic pioneer cells delaminate, subsequently migrate

MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

[†]Present address: Victor Chang Cardiac Research Institute, 384 Victoria Street, Darlinghurst, New South Wales 2010, Australia

⁺Corresponding author. Tel: +612 9295 8535; Fax: +612 9295 8501;

E-mail: geohol@garvan.unsw.edu.au

Submitted 4 April 2003; accepted 15 July 2003

underneath the dermomyotomal epithelium and move anteriorly to occupy a rostral domain (Fig. 1A). These cells then differentiate as mononucleated myofibres that individually span the rostral-to-caudal extent of each somitic segment (Fig. 1B). The myotome is formed as these cells simultaneously differentiate underneath the entire dorsomedial–ventrolateral extent of the dermomyotome (Kahane *et al.*, 1998a).

Fig. 1 | Myoblast migration and myofibre growth of the two models of avian myotome formation. The dermomyotome epithelium (DM) has been lifted, leaving the myotome and the dermomyotome edges, the dorsomedial lip (DML), the ventrolateral lip (VLL), the rostral lip (R) and the caudal lip (C). Blue arrows represent migration of myoblasts, red arrows represent growth of myofibres and black lines represent elongated myofibres. (A–C) In the Kalcheim model (see text for details), the pioneer myoblasts originate along the rostral–caudal extent of the dorsomedial wall, delaminate and migrate to rostral positions (A), and the myofibres then grow in a rostral-to-caudal direction (B). A second wave of fibres arises from all four myotome edges, the myoblasts from the rostral and caudal edges directly generate myofibres, whereas those from the DML and VLL first migrate to the rostral and caudal edges before entering the myotome (C). The second wave causes expansion of the myotome in both the rostrocaudal and dorsoventral directions and also increases the myotome thickness in the transverse plane. (D–F) In the Ordahl model (see text for details), myoblasts enter from the DML along its rostral–caudal extent (D), and myofibres grow out towards the rostral and caudal edges simultaneously from many points along the rostral–caudal extent of the myotome (E). Myoblasts continue to be added from the DML, and the VLL, and older fibres are displaced more laterally (F). NC, notochord; NT, neural tube.

The second wave of myoblast migration results in the expansion of this pre-existing structure. At this stage (Fig. 1C), myotomal growth occurs both in the rostrocaudal and dorsoventral directions and also results in an increase in the thickness of the tissue across the transverse plane; the pioneer cells act as a scaffold for the intercalation of the new cells, which arise from all four dermomyotomal border, or lip, regions (Kahane *et al.*, 1998b; Cinnamon *et al.*, 1999). Myoblasts are added from the rostral and caudal dermomyotome edges, and these cells directly generate myofibres that run in the same direction as the primary pioneer fibres that arose during the first wave (Kahane *et al.*, 1998b). Cells from the DML and the ventrolateral lip (VLL) also contribute to the expansion, but they first delaminate into an intermediate zone between the epithelia and the myotome, and migrate as mesenchymal cells to the rostral and caudal lips of the dermomyotome, before entering the myotome itself. They then differentiate into myofibres and intercalate among the fibres from the first wave (Cinnamon *et al.*, 2001; Kahane *et al.*, 2002). In this way, the myotome is thought to expand along its entirety in the mediolateral direction and, as a consequence, contains both old and young fibres throughout. The cells of the lips continually proliferate and so progressively produce fibres that generate myotomal growth.

During secondary myogenesis, multinucleated fibres are formed by fusion, including some between pioneer and younger cells (Kahane *et al.*, 2002). Myofibres extend directionally from either the rostral or caudal lip region and are anchored at one end as they grow. Kahane and colleagues (2001) also described a third wave of progenitors, which are mitotically active cells that enter the myotome from the rostral and caudal dermomyotome edges. These cells steadily increase in number such that by embryonic day 4 they are estimated to contribute up to 85% of the total myotomal muscle mass.

Ordahl and colleagues have also extensively studied the formation of the myotome in avian embryos and also believe in the importance of the dermomyotomal lips to myotome formation. However, their proposed mechanism of myotome formation differs from the random intercalatory scaffold model of Kalcheim and colleagues. The technique of Dil labelling during chick embryo development was coupled with the generation of chick–quail chimaeras and surgical ablation to track the embryonic origin of myotomal cells (Denetclaw *et al.*, 1997; Denetclaw & Ordahl, 2000; Ordahl *et al.*, 2001; Venters & Ordahl, 2002). On the basis of the results of these experiments, the authors propose that the primary myotome forms as a consequence of the translocation (Fig. 1D) and subsequent elongation (Fig. 1E) of cells from the DML, and grows as older fibres are displaced laterally and new cells are added from the DML. They believe that both the DML and VLL regions contribute cells that translocate directly to the forming myotome, rather than first migrating to the rostral and caudal lips (Fig. 1F). Ordahl and colleagues (2001) have further emphasized the role of the DML in primary myotome formation through transplantation and ablation experiments that showed that cells from the DML are both necessary and sufficient for growth and morphogenesis of the primary myotome. They further showed that cells from other regions, including older regions of the myotome, are not required once the DML activity has started. They concluded that the DML emits two streams of cells: one that translocates to the myotome and one that enters the dermomyotome epithelium. Consequently, the DML region constitutes a

self-renewing 'stem-cell-like' progenitor system, which simultaneously gives rise to cells that contribute to the myotome and the dermomyotome (Venters & Ordahl, 2002). Additionally, they believe that the dorsomedial lip retains the capacity to contribute to all of the cell types of the primary epaxial myotome throughout the embryonic period.

The lack of a consensus about an avian model of myotome formation is further complicated by the results of studies in mouse embryos that used retrospective clonal analysis of LacZ-stained cells to examine the steps in myotomal growth and the separation of the medial epaxial myotome from the lateral hypaxial domain. These analyses show that the fate of medial and lateral precursors is determined even before somite segmentation occurs, and that they exhibit clonal inheritance after segmentation (Eloy-Trinquet *et al.*, 2000; Eloy-Trinquet & Nicolas, 2002a,b). The two separate medial and lateral domains have a striking similarity in terms of their clonal behaviour. Furthermore, this regionalization within the myotome was found to reflect a spatial relationship between precursor populations and the dermomyotome, and was even shown to reflect a regionalization within the DML itself (Eloy-Trinquet & Nicolas, 2002b). This information has led to a model in which there is little cell movement or intercalation, as cells within the dermomyotome give rise directly to the region of the myotome in which they themselves are positioned (Eloy-Trinquet & Nicolas, 2002a). Unfortunately, it is impossible to determine exactly when the observed LacZ-positive myotome progenitors are born, and so it is difficult to compare these studies with those on early chick myotome formation. However, such observations are clearly at odds with any model that proposes extensive cell movements to generate the mature myotome, although it is consistent with earlier observations in the mouse embryo (Sporle, 2001) that, on the basis of gene expression data, the mouse myotome forms three distinct medial–lateral areas. Other authors have indirectly assayed myotome growth in transgenic mouse embryos in which LacZ expression was driven by a muscle-specific transgene and have coupled this with an analysis of the expression of muscle structural genes (Venters *et al.*, 1999). The results suggested that the DML lip, together with rostral and caudal aspects of the medial dermomyotome, are indeed the sources of myotomal growth, and that they take part in a process in which medial addition of new myocytes laterally displaces more mature myocytes (Venters *et al.*, 1999). Despite a lack of evidence to support a random distribution of myotomal precursors, the earliest identifiable differentiating muscle cells of the primary mouse myotome do span the rostral–caudal extent of the somite and have large, centrally located nuclei. This morphology is highly reminiscent of the chick pioneer cells identified by Kalcheim and colleagues (1999).

It is clear, however, that the studies performed to elucidate myotome formation in an amniote species have failed to reach a consensus and have surprisingly resulted in the development of genuinely contradictory models of cellular morphogenesis. Thus, the exact morphogenetic process that muscle precursor cells undergo to form the amniote myotome remains to be resolved.

The fish myotome

Faced with such a bewildering array of conflicting analyses, can some clarity be gained by examining an organism that has a simple, well-studied myotomal structure, in which a consensus about cell migration and muscle formation has been achieved? Analyses

Fig. 2 | Cell migration within the developing zebrafish myotome. **(A)** Adaxial cells arise and differentiate adjacent to the notochord and migrate radially out to the lateral edge. **(B)** More myofibres are added in growth zones at the dorsal and ventral extremes of the myotome. Blue arrows represent migrating muscle cells and red arrows represent growth of muscle fibres along the rostral–caudal extent of the myotome. The dorsal and ventral growth zones are shown in dark green and mature muscle fibres are shown in black.

in zebrafish, which have been aided by the optical clarity of the embryo and the comparative ease of tracing individual cell movements in real time during myotome formation, have afforded such a consensus. In zebrafish, there is no recognizable intermediate epithelial dermomyotomal stage for myotome formation. Indeed, muscle differentiation initiates before segmentation of the epithelial somite within the most medial cells of the presomitic mesoderm, termed the adaxial cells (Devoto *et al.*, 1996; reviewed in Brennan *et al.*, 2002). These early-specified precursors differentiate at their origin, which flanks the notochord, specifically as slow-twitch, myosin heavy chain (MyHC)-expressing muscle cells (Fig. 2A). These then migrate from their origin to form a superficial, subcutaneous layer of muscle cells at the lateral-most extent of the myotome (Devoto *et al.*, 1996; Blagden *et al.*, 1997; Fig. 2A). This medial-to-lateral migration is co-ordinated with the onset of differentiation of the rest of the cells of the myotome,

which occurs behind the differentiating wave of adaxial cell migration. A subset of adaxial cells fails to undergo this migration and remains medial, juxtaposed to the notochord. These cells, known as the muscle pioneer cells, elongate and striate to span the rostral–caudal extent of the forming myotome, and are in fact the first elongating and differentiating cells of the zebrafish myotome. Their non-migratory nature and unique gene expression profiles set these cells apart from other adaxially derived myoblasts. The similarity of morphogenesis of adaxial cells to that of the postulated early pioneer cell model of the avian embryo is reinforced by the fact that avian pioneer cells also specifically express slow MyHC (Kalcheim *et al.*, 1999). Thus, in this context, it would seem that teleost myotome formation most closely resembles a model in which pioneer myoblasts provide a scaffold for future growth. Although the definitive homology between fish and chick pioneer myoblasts is far from established, it is tempting to propose that the amniote myotome evolved from the simple arrangement of the teleost structure.

More recently, the mechanism of myotomal growth has been analysed in zebrafish embryos (Barresi *et al.*, 2001). This has identified growth zones at the dorsal and ventral extremes of the myotomes as the regions where new fibres are added during larval growth (Fig. 2B), which is a process originally defined as stratified hyperplasia in studies of marine teleosts (reviewed in Rowleson & Veggetti, 2001). Furthermore, patterning and specification of the types of fibre that are produced from dorsal–ventral–lip-like regions seems to be independent of signals that operate to pattern the embryonic myotome. This mechanism of myotomal growth resembles the amniote model of myotome formation described by Ordahl and colleagues (2001), in which both the dorsomedial and ventrolateral lips of the chick dermomyotome contribute to myotome formation. It therefore seems that aspects of both models of amniote myotome formation operate within the zebrafish myotome, which suggests that this may also be true for a diverse range of vertebrate species. An issue that must be resolved in the future is how the different mechanisms of growth and patterning that operate in individual organisms are balanced.

Models of limb and fin muscle formation

In amniotes, the hypaxial dermomyotome is also the source of limb muscle precursor cells. Limb muscles exhibit discontinuous development, and are specified initially within the hypaxial dermomyotome, adjacent to the forming limb bud, through the action of as yet unidentified inductive signals. The generation of chimaeras of chick and quail embryos has revealed that the avian limb musculature originates from cells within the lateral half of limb-level somites (Christ *et al.*, 1977; Chevallier *et al.*, 1977; Ordahl & Le Douarin, 1992). These cells are specifically located in the lateral lip of the dermomyotome (Christ & Ordahl, 1995) and characteristically express several different genes, the activities of which are required for aspects of their consequent morphogenesis (Mennerich *et al.*, 1998; Schäfer & Braun, 1999; Dietrich *et al.*, 1999; Mankoo *et al.*, 1999).

Within the dermomyotome, limb muscle precursors undergo an epithelial-to-mesenchymal transition before delaminating from the somites. Cells migrate singly or as small groups into the limb bud, where they proliferate and then differentiate to form the future limb dorsal and ventral muscle masses (Williams & Ordahl, 1994). More recent studies have shown that a similar

process controls the formation of the mouse limb muscle, and a general consensus has emerged that avian and mammalian species generate their appendicular muscles through closely related processes. This assumption has been given further credence by the identification of homologous genes that co-ordinate limb muscle formation within the two groups.

Until recently, the existence of homologous, somitically derived, fin myoblasts had not been documented in any species. In fact, the only detailed analysis of fin muscle formation had come from studies performed at the turn of the nineteenth century, most of which used primitive cartilaginous or chondrichthyan species such as sharks. A detailed characterization of spotted dogfish shark (*Scylliorhinus canicula*) embryos at different stages suggested that pectoral fin muscle is derived from the direct extension of myotome cells into the developing fin bud to form a continuous muscle mass (Dohrn, 1884; Braus, 1899; reviewed in Goodrich, 1958). On the basis of these and other studies, the 'textbook' view has been that all fish generate fin muscle in this manner, and that the tetrapod migratory mode of limb muscle formation evolved to generate the more complex muscle required for limb-dominant locomotion strategies (Kardong, 1995; Amthor *et al.*, 1998). However, zebrafish fate mapping and time-lapse analyses show that teleost fin myoblasts originate from fin-level somites, that these cells delaminate and migrate, and that they also express genes that are specifically expressed in limb muscle precursors within amniote embryos (Neyt *et al.*, 2000; Fig. 3). Collectively, these studies reveal that zebrafish fin muscle morphogenesis uses the same mechanisms as those used by amniote embryos to generate limbs. However, a re-examination of fin

A Chondrichthyan (primitive mode)

B Tetrapods and zebrafish (derived mode)

Fig. 3 | Fin muscle formation. (A) Chondrichthyans use epithelial extensions (arrows) from the myotome (MY) to generate the muscles of the fin (F). (B) Teleosts use an epithelial-to-mesenchymal transition of ventrolateral cells of the myotome to generate the muscles of the fins—a mechanism identical to that used in amniote embryos to generate the limb muscles. c, chondrocytes; NC, notochord; NT, neural tube.

muscle formation in *S. canicula* has confirmed the existence of muscle fibres that are headed by epithelial extensions and extend directly from the body into the developing fin field. Furthermore, these are also deployed to generate the inter-fin-level muscles of the ventral body wall. *S. canicula* somite extensions do not express genes that are common to mesenchymal migrating limb/fin myoblasts in other species, which reinforces the theory that the control selacian fin muscle formation is of a primitive nature.

The comparisons discussed above indicate that the proliferative, migratory and mesenchymal nature of appendicular muscle precursors was generated before the formation of the tetrapod limb. However, amniotes retain the primitive mode of muscle formation for the regions between the limbs (inter-limb level), using structures called epithelial somitic buds to form body wall and intercostal muscles by continuous ventral extension of the lateral dermomyotome (Christ *et al.*, 1983; Christ & Ordahl, 1995; Cinnamon *et al.*, 1999; Denetclaw & Ordahl, 2000; Dietrich *et al.*, 1998; Houzelstein *et al.*, 1999). Furthermore, the lateral somitic buds of inter-limb somites do not express genes that have been specifically implicated in the genetic control of limb myoblast migration. Thus, the inter-limb-level somites of amniotes have morphogenetic and molecular similarity to the fin- and inter-fin-level somites of selacians, suggesting that the mechanisms used to generate hypaxial muscle from these somites are evolutionarily related and represent the primitive condition for somite morphogenesis. This mechanism was genetically altered before the sarcopterygian radiation (Fig. 4), to generate migratory myoblasts within fin-level somites that would evolve to give the appendicular musculature present in bony fishes and tetrapods.

Further analysis must be performed in several species to examine the details of the simple model in which the evolution of migratory appendicular myoblasts occurred before the sarcopterygian radiation. It will be essential to examine more species within distinct vertebrate radiations and to determine exactly how tetrapod limb muscles evolved from the platform of migratory somitically derived sub-populations. The available data do suggest, however, that the zebrafish will be a useful model system in which to analyse the genetic basis of both fin and limb muscle formation, given the shared morphogenetic process that these evolutionarily related cell populations have been shown to undergo.

Conclusions

Recent studies have revealed some of the complex morphogenetic processes that occur within the amniote somite to generate the embryonic myotome. Different experimental approaches have led to the establishment of two models of myotome formation in avian embryos, which on the surface seem mutually exclusive. The first model suggests that waves of myoblast migration, beginning with that of the pioneer myoblasts, generate the myotome. Myotomal growth consequently occurs through random intercalation of subsequent waves of myoblast migration. A second model proposes that the progressive addition of cells delaminating from specific lip regions of the dermomyotome generates the embryonic myotome and that one of these regions, the DML, acts as a source of self-renewing stem-cell progenitors. Elements of both these models seem to operate in the zebrafish myotome, in which the initial stages of myotome formation occur through a process similar to that of avian pioneer myoblast migration, and later growth occurs

Fig. 4 | Vertebrate phylogeny, with an emphasis on extant fish species. Species discussed in the text are indicated by arrowheads. The sarcopterygian radiation is denoted by a thick black line. The red line represents the probable origin of the derived mode of appendicular muscle formation.

through the progressive addition of new fibres at the ends of the forming myotome. These regions seem to be homologous, at least superficially, to the dermomyotomal lip structures postulated to be active in the second model of avian myotome formation. Collectively, these results suggest that it is likely that both mechanisms operate to simultaneously generate myotomal growth and pattern in a wide variety of vertebrate species.

The morphogenetic processes that generate fin and limb muscle are less controversial, and a unified model of appendicular muscle formation has developed. Indeed, most vertebrates appear to use the same mechanism of myoblast delamination and migration in this process. Only primitive, cartilaginous fish use the system of continuous epithelial myotomal extension to generate fin muscle, and this process seems to have been retained in all vertebrates to generate inter-fin/inter-limb hypaxial muscles. A challenge for the future is to determine the molecular basis of these different modes of muscle formation.

ACKNOWLEDGEMENTS

We thank S. Bruce for technical assistance. P.D.C. is supported by grants from the Medical Research Council and the Muscular Dystrophy Campaign UK, and is the recipient of an EMBO Young Investigator Award. G.E.H. is supported by a Howard Florey Fellowship.

REFERENCES

- Amthor, H., Christ, B., Weil, M. & Patel, K. (1998) The importance of timing differentiation during limb muscle development. *Curr. Biol.*, **21**, 642–652.
- Bardeen, C.R. (1900) The development of the musculature of the body wall in the pig. *Johns Hopkins Hosp. Rep.*, **9**, 367–399.
- Barresi, M.J., D'Angelo, J.A., Hernandez, L.P. & Devoto, S.H. (2001) Distinct mechanisms regulate slow-muscle development. *Curr. Biol.*, **11**, 1432–1438.
- Blagden, C.S., Currie, P.D., Ingham, P.W. & Hughes, S.M. (1997) Notochord induction of zebrafish slow muscle is mediated by Sonic Hedgehog. *Genes Dev.*, **11**, 2163–2175.
- Boyd, J.D. (1960) in *Structure and Function of Muscle* (ed. Bourne, G.H.) 63–85. Academic, New York, USA.

- Braus, H. (1899) Beiträge zur entwicklung der muskulatur und des peripheren nervensystems der selachier. *Morphologisches Jahrbuch*, **27**, 501–629.
- Brennan, C., Amacher, S.L. & Currie, P.D. (2002) Pattern formation in zebrafish. Aspects of organogenesis: somitogenesis. *Results Probl. Cell Differ.*, **40**, 271–297.
- Chevallier, A., Kieny, M., Mauger, A. & Sengel, P. (1977) in *Vertebrate Limb and Somite Morphogenesis* (eds Ede, D.A., Hinchcliffe, J.R. & Balls, J.) 421–432. Cambridge Univ. Press, London, UK.
- Christ, B. & Ordahl, C.P. (1995) Early stages of chick somite development. *Anat. Embryol. (Berl.)*, **191**, 381–396.
- Christ, B., Jacob, H. & Jacob, M. (1977) Experimental analysis of the origin of the wing musculature in avian embryos. *Anat. Embryol. (Berl.)*, **150**, 171–186.
- Christ, B., Jacob, H.J. & Jacob, M. (1978) Regional determination of early embryonic muscle primordium. Experimental studies on quail and chick embryos (demonstration). *Verh. Anat. Ges.*, **72**, 353–357.
- Christ, B., Jacob, M. & Jacob, H.J. (1983) On the origin and development of the ventro-lateral trunk musculature in the avian embryo. An experimental and ultrastructural study. *Anat. Embryol. (Berl.)*, **166**, 87–101.
- Cinnamon, Y., Kahane, N. & Kalcheim, C. (1999) Characterisation of the early development of specific hypaxial muscles from the ventrolateral myotome. *Development*, **126**, 4305–4315.
- Cinnamon, Y., Kahane, N., Bachelot, I. & Kalcheim, C. (2001) The sub-lip domain—a distinct pathway for myotome precursors that demonstrate rostral–caudal migration. *Development*, **128**, 341–351.
- Denetclaw, W.F. Jr & Ordahl, C.P. (2000) The growth of the dermomyotome and formation of early myotome lineages in thoracolumbar somites of chicken embryos. *Development*, **127**, 893–905.
- Denetclaw, W.F. Jr, Christ, B. & Ordahl, C.P. (1997) Location and growth of epaxial myotome precursor cells. *Development*, **124**, 1601–1610.
- Devoto, S.H., Melancon, E., Eisen, J.S. & Westerfield, M. (1996) Identification of separate slow and fast muscle precursor cells *in vivo*, prior to somite formation. *Development*, **122**, 3371–3380.
- Dietrich, S., Schubert, F.R., Healy, C., Sharpe, P.T. & Lumsden, A. (1998) Specification of the hypaxial musculature. *Development*, **125**, 2235–2249.
- Dietrich, S., Abou-Rebyeh, F., Brohmann, H., Bladt, F., Sonnenberg-Riethmacher, E., Yamaai, T., Lumsden, A., Brand-Saberi, B. & Birchmeier, C. (1999) The role of SF/HGF and c-Met in the development of skeletal muscle. *Development*, **126**, 1621–1629.
- Dohrn, A. (1884) Die paarigen und unpaaren Flossen der Selachier. *Mitteilungen Zoologischen Station Neapol.*, **5**, 161–195.
- Eloy-Trinquet, S. & Nicolas, J.F. (2002a) Clonal separation and regionalisation during formation of the medial and lateral myotomes in the mouse embryo. *Development*, **129**, 111–122.
- Eloy-Trinquet, S. & Nicolas, J.F. (2002b) Cell coherence during production of the presomitic mesoderm and somitogenesis in the mouse embryo. *Development*, **129**, 3609–3619.
- Eloy-Trinquet, S., Mathis, L. & Nicolas, J.F. (2000) Retrospective tracing of the developmental lineage of the mouse myotome. *Curr. Top. Dev. Biol.*, **47**, 33–80.
- Goodrich, E.S. (1958) *Studies on the Structure and Development of Vertebrates*, Vol. 1. Dover Publications, New York, USA/Constable & Co. Ltd, London, UK.
- Hamilton, H.L. (1952) *Lille's Development of the Chick. An Introduction to Embryology*. Holt, Reinhardt & Winston, New York, USA.
- His, W. (1888) Untersuchungen über die erste anlage des wirbeltier leibes. Die erste entwicklung des hühnchens im ei. *Leipzig*, **16**, 237.
- Houzelstein, D., Auda-Boucher, G., Cheraud, Y., Rouaud, T., Blanc, I., Tajbakhsh, S. & Buckingham, M.E. (1999) The homeobox gene *Msx1* is expressed in a subset of somites, and in muscle progenitor cells migrating into the forelimb. *Development*, **126**, 2689–2701.
- Kahane, N., Cinnamon, Y. & Kalcheim, C. (1998a) The origin and fate of pioneer myotomal cells in the avian embryo. *Mech. Dev.*, **74**, 59–73.
- Kahane, N., Cinnamon, Y. & Kalcheim, C. (1998b) The cellular mechanism by which the dermomyotome contributes to the second wave of myotome development. *Development*, **125**, 4259–4271.
- Kahane, N., Cinnamon, Y., Bachelot, I. & Kalcheim, C. (2001) The third wave of myotome colonisation by mitotically competent progenitors: regulating the balance between differentiation and proliferation during muscle development. *Development*, **128**, 2187–2198.
- Kahane, N., Cinnamon, Y. & Kalcheim, C. (2002) The role of cell migration and myofibre intercalation in patterning formation of the postmitotic myotome. *Development*, **129**, 2675–2687.
- Kalcheim, C., Cinnamon, Y. & Kahane, N. (1999) Myotome formation: a multistage process. *Cell Tissue Res.*, **296**, 161–173.
- Kardong, K.V. (1995) *Vertebrates, Comparative Anatomy, Function, Evolution*. William C. Brown, Oxford, UK.
- Langman, J. & Nelson, G.R. (1968) A radioautographic study of the development of the somite in the chick embryo. *J. Embryol. Exp. Morphol.*, **19**, 217–226.
- Mankoo, B.S., Collins, N.S., Ashby, P., Grigorieva, E., Pevny, L.H., Candia, A., Wright, C.V.E., Rigby, P.W.J. & Pachnis, V. (1999) *Mox-2* is a component of the genetic hierarchy controlling limb muscle development. *Nature*, **400**, 69–73.
- Mennerich, D., Schäfer, K. & Braun, T. (1998) Pax-3 is necessary but not sufficient for *lhx1* expression in myogenic precursor cells of the limb. *Mech. Dev.*, **73**, 147–158.
- Neyt, C., Jagla, K., Thisse, C., Thisse, B., Haines, L. & Currie, P.D. (2000) Evolutionary origins of vertebrate appendicular muscle. *Nature*, **408**, 82–86.
- Ordahl, C.P. & Le Douarin, N.M. (1992) Two myogenic lineages within the developing somite. *Development*, **114**, 339–353.
- Ordahl, C.P., Berdugo, E., Vinters, S.J. & Denetclaw, W.F. Jr (2001) The dermomyotome dorsomedial lip drives growth and morphogenesis of both the primary myotome and dermomyotome epithelium. *Development*, **128**, 1731–1744.
- Remak, R. (1855) *Untersuchungen über die entwicklung der wirbelthiere*. G. Reimer, Berlin, Germany.
- Rowlerson, A. & Veggetti, A. (2001) in *Muscle Development and Growth* (ed. Johnston, I.A.) 103–140. Academic, London, UK.
- Schäfer, K. & Braun, T. (1999) Early specification of limb muscle precursor cells by the homeobox gene *Lbx1*. *Nature Genet.*, **23**, 213–216.
- Sporle, R. (2001) Epaxial–adaxial–hypaxial regionalisation of the vertebrate somite: evidence for a somitic organiser and a mirror-image duplication. *Dev. Genes Evol.*, **211**, 198–217.
- Vinters, S.J. & Ordahl, C.P. (2002) Persistent myogenic capacity of the dermomyotome dorsomedial lip and restriction of myogenic competence. *Development*, **129**, 3873–3885.
- Vinters, S.J., Thorsteinsdottir, S. & Duxson, M.J. (1999) Early development of the myotome in the mouse. *Dev. Dyn.*, **216**, 219–232.
- Williams, B.A. & Ordahl, C.P. (1994) Pax-3 expression in segmental mesoderm marks early stages in myogenic cell specification. *Development*, **120**, 785–796.
- Williams, L.W. (1910) The somites of the chick. *Am. J. Anat.*, **11**, 55–100.

Georgina E. Hollway

Peter D. Currie is the recipient of an EMBO Young Investigator Award