# Migration of Myogenic Cells in the Developing Limb

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**Abstract**

Myogenic cells migrate into the limb from the somitic mesoderm, taking up positions in the dorsal and ventral compartments before differentiating into the limb musculature. This process is influenced by the tissues of the limb in a number of ways. Cells of the lateral part of the somitic dermomyotome are induced to express a migratory myogenic phenotype by a diffusible signal originating in the limb bud mesoderm. The limb and body wall musculature are entirely derived from cells of this migratory myogenic lineage. Components of the extracellular matrix of the limb mesoderm, such as fibronectin, facilitate myogenic cell migration by providing sites for adhesion and directed migration. Differences in the composition of the matrix along the proximo-distal axis make the distal mesoderm more attractive for migrating myogenic cells. Anterior-posterior movement of myogenic cells within the limb bud is highly restricted, resulting in a regionalised contribution of cells to the limb musculature based on somitic origin. Lastly, there is a process that excludes myogenic cells from the most distal region of the limb bud mesoderm; a similar process is involved in the aggregation of myogenic cells into the dorsal and ventral premuscle masses. These findings illustrate the high degree of control over myogenic cell migration mediated by both diffusible factors and the extracellular matrix in the developing limb mesenchyme.

**Key words:** myogenesis, cell migration, extracellular matrix, limb bud, somites.

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The limb musculature is derived from myogenic cells that migrate from the lateral half of the dermomyotome of the somite. Migration of myogenic cells from those somites medial to the limb bud starts as soon as the bud begins to form. The cells aggregate within the limb bud into two premuscle masses, which are the sites of the initial stages of myogenic differentiation. Studies in recent years have focussed on four broad issues concerned with myogenic cell migration in the limb: the migratory myogenic phenotype, the activity of the extracellular matrix in facilitating myogenic cell migration, the restriction of anterior-posterior movement of myogenic cells during the migration phase and the exclusion of myogenic cells from such regions of the limb bud as the progress zone and sites of skeletal differentiation. This review brings together the salient findings supporting the role played by these four factors in myogenic cell migration.

## The Migratory Myogenic Cell Lineage

Early findings from chick-quail grafting studies led to the notion of two lineages of skeletal myogenic cells both originating from the dermomyotome; a migratory lineage that gives rise to the body wall and limb musculatures and a myotomal lineage that differentiates *in situ* to form the back muscles [12, 13]. This view is supported by more recent findings on the fate of the medial and lateral somite halves [29]. It was shown that cells from the lateral half of the dermomyotome migrate to the developing limb and body wall and form the skeletal musculature of those structures while the population of medial myogenic cells initially forms the myotome, then differentiates into the back muscles. These lineages differ in their requirements for signals from the neural tube; limb myogenesis occurs in the absence of the neural tube but myotome myogenesis does not [4]. The Pax-3 transcriptional regulator is also expressed at higher levels only in the lateral dermomyotome and in limb myogenic cells [40]. Moreover, mouse embryos homozygous for a mutant allele of the Pax-3 gene have reduced limb musculature [5, 16] but normal back muscles. This finding suggests that Pax-3 expression is required for efficient myogenic cell migration but not the differentiation of the medial myogenic cell population into the myotome and thence into the back musculature.

Divergence of the two myogenic lineages may result from the action of a diffusible signal secreted by the presumptive limb mesoderm. This hypothesis contends

that signalling molecules from the limb bud mesoderm repress the program of myogenic differentiation and induce cells of the lateral dermomyotome to migrate. Thus, the myotomal lineage may be viewed as the 'default' lineage and the migratory phenotype only arises because of local signalling from the lateral or the limb bud mesoderm. Support for this notion has come from evidence for repressed myogenic differentiation in cells of the putative myotome by ectopic grafts of lateral plate mesoderm dorsal to the somitic mesoderm. The grafts repressed the expression of the myogenic transcriptional regulators MyoD, myogenin and Myf-5 and upregulated the expression of Pax-3 in the medial half of the dermomyotome [30]. Further support for a migratory myogenic lineage has come from the finding that scatter factor/hepatocyte growth factor (SF/HGF) has a crucial role in determination the migratory myogenic phenotype. SF/HGF is expressed in the early limb bud before the commencement of myogenic cell migration [2, 34, 38]. Signalling by the growth factor is transmitted via a receptor, c-met [3, 39], which is expressed in the dermomyotome and in cells within the limb bud during the period of myogenic cell migration [34]. Embryos fail to develop a limb musculature when c-met is inactivated by homologous recombination [2]. Moreover, Pax-3 and myogenin expressing cells are absent in the limb in these embryos during the period of myogenic migration, suggesting that SF/HGF signalling is required for the migration of myogenic cells into the limb. This role for SF/HGF is consistent with *in vitro* experiments in which SF/HGF was shown to induce cells in epithelial sheets to dissociate and migrate [9, 17, 36]. The findings that the lateral mesoderm represses myogenesis and that SF/HGF signalling by the limb is required for myogenic cell migration provide strong support for the notion of a migratory myogenic phenotype.

## Role of the Extracellular Matrix in Facilitating Myogenic Cell Migration

Extracellular matrix molecules almost certainly play a central role in facilitating the migration of myogenic cells in the limb. Some molecules form part of the substrate that myogenic cells need for movement. Other molecules may help direct myogenic cells in the distal direction. Evidence suggests movement of the cells occurs by active locomotion rather than via a passive translocatory process [37].

Fibronectin and laminin are extracellular matrix molecules that play an adhesion role in myogenic cell migration. They are both uniformly distributed in the limb mesoderm during the period of myogenic cell migration [11, 18], but this expression pattern changes during the formation of the two premuscle masses. Fibronectin and laminin substrates have both been shown to promote myogenic cell migration *in vitro* [28]. Moreover, injections into the chick wing of the antibody to fibronectin's cell attachment domain prevents grafted quail myogenic cells migrating from the site of implantation [8]. The latter study demonstrates that adhesion to the extracellular matrix is essential to myogenic cell migration in the limb.

Hyaluronate is present in high quantities in the peripheral region of chick wing buds at the time of myogenic cell migration [23, 33]. Injections of hyaluronate into the chick wing mesoderm just proximal to grafts of quail wing tissue result in atypical migration of quail myogenic cells in the proximal direction [25]. It was suggested that hyaluronate widens intercellular spaces, allowing increased penetration by migrating myogenic cells. However this effect of hyaluronate may be over-ridden by other determining factors since it is highly expressed in regions that are free of myogenic cells such as the progress zone and the ectoderm/mesoderm interface [33].

Temporal and regional differences in the extracellular environment of limb mesoderm have an important role in myogenic cell migration. A study by Brand-Saberi and co-workers [7] showed that developmentally-younger limb mesoderm tissue has an extracellular environment that is more attractive for myogenic cell migration than older tissue. Myogenic cells migrate from grafted quail wing bud tissue into host chick wing bud tissue if the host tissue is younger, but not if the host is older. Attractiveness to myogenic cells also changes along the proximo-distal axis. Distal migration of myogenic cells from quail wing tissue implants occurs if they are grafted into regions that are distal to their origin [7], suggesting that a gradient of attractiveness for myogenic cells exists along the proximo-distal axis of the limb bud. The basis for such a gradient may reside with differences in the expression of extracellular matrix molecules along the long axis of the limb. One molecule of this type is the presently uncharacterised antigen recognized by antibody JUV97 [6]. The antibody shows strongest immuoreactivity in more distal compared with proximal regions of the limb. Moreover, injection of the antibody into the chick limb stops myogenic cell migration, suggesting a role for graded cues in myogenic cell migration in the limb.

## Restriction of the Anterior-Posterior Movement of Migrating Myogenic Cells

Evidence for the restriction of anterior-posterior movement during the period of myogenic cell migration has come from studies of the contribution of single somites to the limb musculature, and from a study of the distribution in the limb bud of migrating myogenic cells derived from pairs of labelled somites. Studies that investigated the fate of cells from individual somitic levels in the chick wing [1] and leg [26] musculature found that limb muscles are derived from a restricted pool of 5 or 6 somites and that cells derived from each somite contribute to only a subset of limb muscles in a topographic manner consistent with their anterior-posterior position. Thus, somites at the anterior end of the somite pool contribute cells to muscles located anteriorly in the limb while more posterior somites contribute to posterior muscles in the limb. The somitic contributions to specific regions within some individual

muscles also appear to be topographically ordered [26], although the contributions of each somite overlap. The pattern of somitic contribution to the limb musculature results from the topographically-segregated migration of myogenic cells [15]; the findings suggest a severe restriction in the anterior-posterior movement of migrating myogenic cells.

The highly directed lateral migration of somitic cells into the limb is not dependent on positional information associated with the cells. Christ and co-workers [11] were first to describe the normal development of limb muscles after the replacement of brachial somites with non-brachial somites. Similar findings were reported by others [10, 22, 26], that is, grafted somites contributed to the limb musculature in accord with their new position, not their position of origin. These results showed that the limb mesoderm cannot recognise the somitic origin of myogenic cells when they migrate into the limb. The cells are perhaps physically restricted from straying off their lateral course of migration and 'channelled' in a distal direction. Krenn and Wachtler [24] grafted quail wing bud tissue proximal to rotated blocks of chick wing tissue; they found that 90° and 180° rotations prevented the migration of grafted myogenic cells after stage 24 [19] of embryonic development. At earlier stages, migration was not impaired by mesoderm rotations, however, this may be due to redetermination of the rotated block of wing bud according to its new position in the limb, as was found with similarly aged grafts in an earlier study [35].

## Exclusion of Myogenic Cells From Regions of the Developing Limb

Myogenic cells are excluded from certain regions of the limb bud during the migration phase. The most distal portion of the limb bud mesoderm, known as the progress zone, has been shown to be free of myogenic cells in a number of studies [21, 27, 31, 32]. Grafting of the progress zone lateral to the somitic mesoderm in chick embryos prevents migration of somitic cells into the developing limb bud [20]. This finding suggests that the progress zone is a very unfavourable environment for migrating myogenic cells, but the basis of this property is not known. Another process by which myogenic cells are excluded from limb bud tissue is the progressive restriction of myogenic cells to the dorsal and ventral premuscle masses before the formation of the adult musculature [21, 27, 32]. This phenomenon is due to the differentiation of the core of the limb bud into skeletal elements which appear to be strongly repellent to myogenic cells.

## Conclusions

All aspects of myogenic cell migration are controlled by molecules expressed in the limb mesoderm. At least two different classes of molecules appear to control myogenic cell migration: diffusible factors that induce and maintain the migratory myogenic phenotype and prevent myogenesis from starting, and structural components of the extracellular matrix. The latter class play a number of roles in myogenic cell migration by making the distal limb mesoderm more attractive to migrating myogenic cells than the proximal mesoderm, restricting anterior-posterior movement of the migrating cells, and excluding myogenic cells from the progress zone and areas of skeletal differentiation. The only role the somitic mesoderm plays in the whole process is to provide the myogenic cells themselves.

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## References

[1] Beresford B: Brachial muscles in the chick embryo: the fate of individual somites. *J Embryol Exp Morph* 1983; 77: 99-116.

[2] Bladt F, Riethmacher D, Isenmann S, Aguzzi A, Birchmeier C: Essential role for the c-met receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 1995; 376: 768-771.

[3] Bottaro DP, Rubin JS, Faletto DL, Chan AM-L, Kmecik TE, Vande Woude GF, Aaronson SA: Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Nature* 1991; 251: 802-804.

[4] Bober E, Brand, Saberi B, Ebensperger C, Wilting J, Balling R, Paterson BM, Arnold HH, Christ B: Initial steps of myogenesis in somites are independent of influence from axial structures. *Development* 1994; 120: 3073-82.

[5] Bober E, Franz T, Arnold H-H, Gruss P, Tremblay P: Pax-3 is required for the development of limb muscles: a possible role for the migration of dermomyotomal muscle progenitor cells. *Development* 1994; 120: 603-612.

[6] Brand-Saberi B, Christ B: Inhibition of myogenic cell migration by the application of antibodies raised against limb bud mesenchyme, in Fallon JF, Goetinck PF, Kelly RO, Stocum DL (eds): *Limb Development and Regeneration.* New York, Wiley-Liss, 1993, pp 541-557.

[7] Brand-Saberi B, Krenn V, Christ B: The control of directed myogenic cell migration into the avian limb bud. *Anat Embryol* 1989; 180: 555-566.

[8] Brand-Saberi B, Krenn V, Grim M, Christ B: Differences in the fibronectin-dependence of migratory cell populations. *Anat Embryol* 1993; 187: 17-26.

[9] Brinkmann V, Foroutan H, Sachs M, Weidner KM, Birchmeier W: Hepatocyte growth factor scatter factor induces a variety of tissue-specific morphogenic programs in epithelial cells. *J Cell Biol* 1995; 131: 1573-1586.

[10] Butler J, Cosmos E, Cauwenbergs P: Positional signals: evidence for a possible role in muscle fibre-type patterning of the embryonic avian limb. *Development* 1988; 102: 763-772.

[11] Christ B, Christ M, Jacob H-J: Zur Frage der regionalen Determination der fruhembryonalen Muskelanlagen Experimentelle Untersuchungen and Wachtel- und Hühnerembryonen. *Verh Anat Ges* 1978; 72: 353-357.

[12] Christ B, Christ M, Jacob H-J: On the origin of the ventrolateral abdominal muscles in the avian embryo. *Anat Embryol* 1983; 166: 87-101.

[13] Christ B, Jacob H-J, Christ M: Über den Ursprung der Flügelmuskulatur. Experimentelle Untersuchungen mit Wachtel- und Hühnerembryonen. *Experientia* 1974; 30: 1446-1449.

[14] Dessau W, Von der Maek H, Von der Mark K, Fischer S: Changes in the patterns of collagens and fibronectin during limb bud chondrogenesis. *J Embryol Exp Morph* 1980; 57: 51-60.

[15] Ewan KBR, Everett AW: A quantitative study of the segmental distribution of somitic cells in the developing chick limb bud using laser-scanning confocal microscopy. *J Histochem Cytochem* 1996; 44: 347-355.

[16] Franz T Kothary R, Surani MAH, Halata Z, Grim M: The Splotch mutation interferes with muscle development in the limbs. *Anat Embryol* 1993; 187: 153-160.

[17] Gherardi E, Gray J, Stoker, Perryman M, Furlong R Purification of scatter factor, a fibroblast-derived basic protein that modulates epithelial interactions and movement. *Proc Natl Acad Sci(USA)* 1989; 86: 5844-5848.

[18] Godfrey EW, Siebenlist RE, Wallskog PL, Watters LM, Bolender DL, Yorde DE: Basal lamina components are concentrated in premuscle masses and at early acetylcholine receptor clusters in the chick embryo hindlimb muscles. *Dev Biol* 1988; 130: 471-486.

[19] Hamburger V, Hamilton HL: A series of normal stages in the development of the chick embryo. *J Morph* 1951; 88: 49-92.

[20] Hayashi K, Ozawa E: Myogenic cell migration from somites is induced by tissue contact with medial region of the presumptive limb mesoderm in chick embryos. *Development* 1995; 121: 661-669.

[21] Hayashi K, Ozawa E: Vital labelling of somite derived myogenic cells in the chicken limb bud. *Wilhelm Roux's Arch Devl Biol* 1991; 200: 188-192.

[22] Keynes RJ, Stirling RV, Stern CD, Summerbell DS: The specificity of motor innervation of the chick wing does not depend on the segmental origin of muscles. *Development* 1987; 99: 565-575.

[23] Kosher RA, Walker KH, Ledger PW: Temporal and spatial distribution of fibronectin during development of the embryonic chick limb bud. *Cell Differentiation* 1982; 11: 217-228.

[24] Krenn V, Wachtler F: The orientation of the wing mesenchyme influences the direction of the migration of myoblasts in the avian embryonic wing bud. *AnatEmbryol* 1990; 181: 453-460.

[25] Krenn V, Brand-Saberi B, Wachtler F: Hyaluronate influence on myoblast migration. *Am J Anat* 1991; 192: 400-406.

[26] Lance-Jones C: The somitic level of origin in embryonic chick hindlimb muscles. *Dev Biol* 1988; 126: 394-407.

[27] Newman SA, Pautou M-P, Kieny M: The distal boundary of myogenic primordia in chimeric avian limb buds and its relation to an accessible population of cartilage progenitor cells. *Dev Biol* 1981; 84: 440-448.

[28] Ocalan M, Goodman SL, Kuhl U, Hauschka SD, Von der Mark K: Laminin alters cell shape and stimulates motility and proliferation of murine skeletal myoblasts. *Dev Biol* 1988; 125: 158-167.

[29] Ordahl CP, Le Douarin NM: Two myogenic lineages within the developing somite. *Development* 1992; 114: 339-353.

[30] Pourquie O, Coltey M, Breaty M, Le Douarin NM: Control of somite patterning by signals from the lateral plate. *Proc Natl Acad Sci (USA)* 1995; 92: 3219-3223.

[31] Rutz R, Hauschka SD: Spatial analysis of limb bud myogenesis: a proximodistal gradient of muscle colony-forming cells in chick embryo leg buds. *Dev Biol* 1982; 90: 399-411.

[32] Schramm C, Solursh M: The formation of premuscle masses during chick wing development. *Anat Embryol* 1990; 182: 235-247.

[33] Singley CT, Solursh M: The spatial distribution of hyaluronic acid and mesenchymal condensation in the embryonic chick wing. *Dev Biol* 1981; 84: 102-120.

[34] Sonnenberg E, Meyer D, Weidner KM, Birchmeier C: Scatter factor/hepatocyte growth factor and its receptor, the c-met tyosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. *J Cell Biol* 1993; 123: 223-235.

[35] Stark RJ, Searls RL: A description of chick wing bud development and a model of limb morphogenesis. *Dev Biol* 1973; 33: 138-157.

[36] Stoker M, Gherardi E, Perryman M, Gray J Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. *Nature* 1987; 327: 239-242.

[37] Sze L-Y, Lee KKH, Webb SE, Li Z, Paulin D: Migration of myogenic cells from the somites to the fore-limb buds of developing mouse embryos. *Developmental Dynamics* 1995; 203: 324-336.

[38] Thery C, Sharpe MJ, Batley SJ, Stern CD, Gherardi E: Expression of HGF/SF, HGA/MSP and c-met suggests new functions during early chick development. *Developmental Genetics* 1995; 17: 90-101.

[39] Weidner KM, Sachs M, Birchmeier W: The *Met* receptor tyrosine kinase transduces motility, proliferation and morphogenetic signals of scatter factor/hepatocyte growth factor in epithelial cells. *J Cell Biol* 1993; 121: 145-154

[40] Williams BA, Ordahl CP: Pax-3 expression in segmental mesoderm marks early stages in myogenic cell specification. *Development* 1994; 120: 785-796.