Development 133, 2897-2904 (2006) doi:10.1242/dev.02464

Regulation of ectodermal Wnt6 expression by the neural tube is transduced by dermomyotomal Wnt11: a mechanism of dermomyotomal lip sustainment

Poongodi Geetha-Loganathan, Suresh Nimmagadda, Ruijin Huang, Bodo Christ* and Martin Scaal

Ectodermal Wnt6 plays an important role during development of the somites and the lateral plate mesoderm. In the course of development, *Wnt6* expression shows a dynamic pattern. At the level of the segmental plate and the epithelial somites, *Wnt6* is expressed in the entire ectoderm overlying the neural tube, the paraxial mesoderm and the lateral plate mesoderm. With somite maturation, expression becomes restricted to the lateral ectoderm covering the ventrolateral lip of the dermomyotome and the lateral plate mesoderm. To study the regulation of *Wnt6* expression, we have interfered with neighboring signaling pathways. We show that Wnt1 and Wnt3a signaling from the neural tube inhibit *Wnt6* expression in the medial surface ectoderm via dermomyotomal Wnt11. We demonstrate that Wnt11 is an epithelialization factor acting on the medial dermomyotome, and present a model suggesting Wnt11 and Wnt6 as factors maintaining the epithelial nature of the dorsomedial and ventrolateral lips of the dermomyotome, respectively, during dermomyotomal growth.

KEY WORDS: Chick embryo, Somite, Neural tube, Dermomyotome, *Wnt6*, *Wnt11*

INTRODUCTION

During the development of the avian embryo, the paraxial mesoderm gives rise to segmental epithelial spheres called somites. The ventral part of the somites subsequently becomes mesenchymal to form the sclerotome, which gives rise to the axial skeleton (Christ et al., 2004). The dorsal part of the somite remains epithelial and becomes the dermomyotome, which gives rise to dermis and muscle. In the course of dermomyotomal differentiation, the dorsomedial and ventrolateral lips of the dermomyotome (DML and VLL, respectively) remain epithelial growth zones (Ordahl et al., 2001), whereas the central dermomyotome (CD) de-epithelializes to give rise to the dorsal dermis and subcutis as well as muscle (Gros et al., 2005; Relaix et al., 2005; Ben-Yair and Kalcheim, 2005) (reviewed by Scaal and Christ, 2004).

Signals from adjacent tissues play important roles in the patterning of somites along the dorsoventral and mediolateral axes. Commitment of cells in somites occurs after somite formation in response to external cues (Aoyama and Asamoto, 1988; Ordahl and Le Douarin, 1992; Christ et al., 1992). Several lines of evidence indicate that Shh secreted from the notochord and floor plate acts as a ventralizing and medializing signal (Fan and Tessier-Lavigne, 1994; Johnson et al., 1994; Chiang et al., 1996; Kos et al., 1998). By contrast, signals from the surface ectoderm and the dorsal neural tube induce the formation and/or maintenance of the dermomyotome (Brand-Saberi et al., 1993; Pourquié et al., 1993; Kuratani et al., 1994; Fan and Tessier-Lavigne, 1994; Spence et al., 1996) (reviewed by Scaal and Christ, 2004).

The Wnt family of secreted proteins play many roles during vertebrate development, including cell fate choice, proliferation and survival (Dickinson and McMahon, 1992; Parr and McMahon, 1994; Cadigan and Nusse, 1997; Huelsken and Birchmeier, 2001). In somite development, Wnt1 and Wnt3a have been shown to be required for the development of the medial and dorsal regions of the somites, as well as to induce myogenesis (Marcelle et al., 1997; Ikeya and Takada, 1998; Wagner et al., 2000). Moreover, the epithelial structure of the newly formed somite, as well as that of the dermomyotome, are influenced by Wnts, which have been suggested to induce an epithelial morphology via β-catenin (Hinck et al., 1994; Gumbiner, 1996; Linker et al., 2005).

A member of this family, *Wnt6*, has been cloned in different species, including human, mouse, *Drosophila*, *Xenopus*, *Amphioxus* and chick, and its expression pattern has been described (Cauthen et al., 2001; Janson et al., 2001; Schubert et al., 2001; Itaranta et al., 2002; Schubert et al., 2002; Rodriguez-Niedenführ et al., 2003; Loganathan et al., 2005). In avian embryos, at the level of the segmental plate and the epithelial somite, *Wnt6* is expressed in the entire ectoderm overlying the neural tube, the paraxial mesoderm and the LPM. Following somite compartmentalization, which leads to the formation of the dermomyotome and the sclerotome, *Wnt6* expression ceases in the medial aspect and becomes restricted to the lateral ectoderm overlying the LPM.

Wnt11 is a member of the non-canonical type of Wnt signaling. Recently, it has been shown to also participate in the canonical Wnt pathway (Tao et al., 2005). In chick, it is expressed in the dorsomedial lip (DML) of matured somites (Marcelle et al., 1997), whereas in zebrafish, Wnt11 is known to be involved in convergence-extension-movements during gastrulation (Heisenberg et al., 2000). The function of Wnt11 in the avian dermomyotome remains unknown.

In this study we examined the regulation of the dynamic pattern of *Wnt6* expression in the avian embryonic ectoderm. We show that *Wnt6* expression in the surface ectoderm becomes downregulated by Wnt1 and Wnt3a signaling from the neural tube. We present evidence that this inhibiting action of the neural tube is mediated by Wnt11 in the DML, thus providing evidence of a patterning influence of neural tube and paraxial mesoderm on the surface ectoderm. Our results suggest that after the lateralization of Wnt6, Wnt11 takes over the function of

Institute of Anatomy and Cell Biology, Department of Molecular Embryology, University of Freiburg, Albertstrasse 17, D-79104 Freiburg, Germany.

*Author for correspondence (e-mail: bodo.christ@anat.uni-freiburg.de)

Accepted 30 May 2006

Wnt6 to maintain the epithelial state of the medial region of the dermomyotome. We present a model suggesting that Wnt11 in the DML, and Wnt6 in the VLL, maintain the epithelial character of the dermomyotomal margins to enable ongoing growth of the dermomyotomal sheet during epaxial and hypaxial myogenesis.

MATERIALS AND METHODS

Preparation of embryos

Fertilized chicken and quail eggs were incubated at 38°C, and the embryos were staged according to Hamburger and Hamilton (Hamburger and Hamilton, 1951).

Separation of somites from axial organs

A longitudinal slit was made between neural tube and adjacent somites [between somites 12-17 (somite stages V-XI), i.e. corresponding to a length of 4-6 somites] on one side of HH stage 14 chick embryos. An aluminium foil barrier was inserted into the slit and the embryo reincubated from 12 to 18 hours and then processed for in situ hybridization.

Removal of neural tube

Portions of unilateral halves or the whole neural tube of HH stage 12-14 chick embryos were removed at the level of epithelial somites (I-IV) just prior to their maturation as previously described (Christ et al., 1992). Embryos were reincubated from 12 to 18 hours, and then processed for in situ hybridization.

Removal of dermomyotome

Whole dermomyotomes or medial dermomyotomes (of somites 10-12, at somite stages V-X) of HH stage 12-13 chick embryos were removed at the level of matured somites. Embryos were reincubated from 12 to 18 hours and then processed for in situ hybridization.

Grafting of medial dermomyotomal lip

Medial lips of dermomyotomes from HH stage 13-14 chick or quail embryos were grafted to the segmental plate of chick embryos of HH stage 11-12. Embryos were reincubated for 8-10 hours, fixed and processed for in situ hybridization and immunohistochemistry.

Cell injection

Wnt3a- and Wnt1-expressing cells were a gift from Andreas Kispert (Medizinische Hochschule Hannover, Germany). CHO B3 cells expressing Noggin protein and DHFR control CHO cells were kindly provided by Richard Harland (University of California at Berkeley). Cell lines were cultured as described elsewhere (Lamb et al., 1993). Confluent cultures were harvested, cells were washed in phosphate-buffered saline (PBS), pelleted and resuspended in a minimal volume of medium. For cell injection, the ectoderm (at the level of somite I-V of HH stage 13-14 embryos) was punctured with a tungsten needle. With the help of a blunt glass needle, a tunnel was made below the ectoderm and concentrated cell suspensions were locally applied with a micropipette along the length of the tunnel. For some embryos, noggin-expressing cells were injected into the neural tube at the level of the epithelial somites. Embryos were reincubated from 12 to 18 hours, processed for whole-mount in situ hybridization. Control cells showed no effect on target genes expression (not shown).

Electroporation of Wnt11 and dnWnt11 RCAS

Wnt11 and dnWnt11 RCAS constructs were kindly provided by Philippa Francis-West (Kings College, London) (Anakwe et al., 2003). The electroporation procedures and equipment were used as described by Scaal et al. (Scaal et al., 2004). Electroporation was performed at the level of epithelial somites (I-IV) of HH stage 12-15 chick embryos. Constructs were co-electroporated with GFP plasmids, the latter electroporated alone were used as a control. Embryos were reincubated from 12 to 16 hours, photographed using a fluorescence microscope to visualize the localization of the plasmid, and then processed for whole-mount in situ hybridization.

In situ hybridization

Embryos were fixed overnight at 4°C in 4% PFA. The embryos were washed in PBT, dehydrated in methanol and stored at 4°C. Whole-mount in situ hybridization was performed as described by Nieto et al. (Nieto et al., 1996). Selected stained embryos were embedded in 4% agar and sectioned with a vibratome at 50 μm.

The following probes were used in this study: chick *Wnt11* (1000 bp; Christophe Marcelle, Marseille); *Pax1* (1.5 kb insert cloned into pBlusescript II KS; Cecilia Ebensperger, Freiburg); *Pax3* (a 1543 bp insert cloned in to pGEM 72f; Marianne Bronner-Fraser, Pasadena); full-length *Paraxis* clone was a gift from Prof. Eric Olson (Dallas). For chick *Wnt6*, we used the cloned *Wnt6* 1500 bp fragment (Rodriguez-Niedenführ et al., 2003), as a template. Sense and antisense riboprobes were labeled with digoxigenin RNA labeling kit as recommended (Boehringer, Mannheim, Germany).

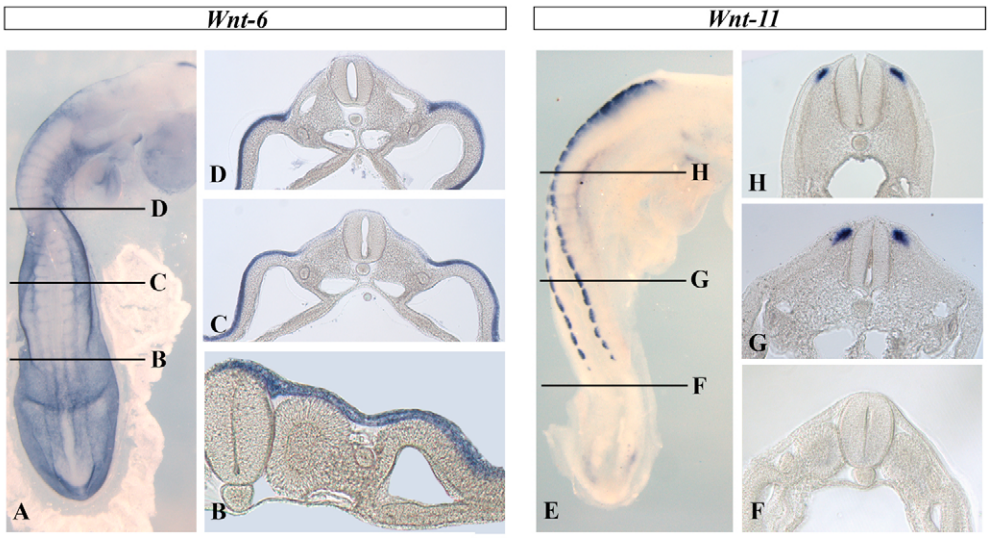

Fig. 1. *Wnt11* expression in the DML and *Wnt6* expression in the ectoderm overlying the somites are mutually exclusive. (**A**,**E**) Whole-mount in situ hybridization of HH stage 15 chick embryos hybridized with Wnt6 (A) and Wnt11 (E) probes. (**B-D**) Transverse sections at different anteroposterior levels of the embryo shown in A. (B) Section at the level of an epithelial somite. *Wnt6* expression in the ectoderm overlying the neural tube, the paraxial mesoderm and the lateral plate mesoderm. (C,D) The somite has compartmentalized and the dermomyotome and the sclerotome are visible. *Wnt6* expression is restricted to the ectoderm overlying the lateral lip of the dermomyotome and the lateral plate mesoderm. (**F-H**) Transverse sections of the embryo shown in E at the AP levels indicated. At the level of the presomitic mesoderm (not shown) and the epithelial somite (F), *Wnt11* is not expressed. *Wnt11* expression starts after compartmentalization of the somite into dermomyotome and sclerotome, with strong expression in the dorsomedial lip of the dermomyotome (G,H).

Immunohistochemistry on whole mounts for the detection of quail cells

Selected embryos after in situ hybridization were used for immunohistochemistry, fixed overnight in 4% paraformaldehyde (PFA), washed in PBS. Following a brief wash in PBS, embryos were incubated overnight with QCPN (anti-quail antibody, DSHB). Embryos were extensively washed in PBS and then incubated overnight in secondary antibody (Cy3-conjugated goat anti-mouse IgG antibody; Jackson ImmunoResearch, 1:100, in PBS). Subsequently, embryos were washed in PBS and stored in 4% PFA.

RESULTS

Wnt signaling from the neural tube restricts *Wnt6* expression to the lateral ectoderm

In order to investigate the regulation of *Wnt6* expression, we re-examined the *Wnt6* expression pattern in the ectoderm during somite development. We found that during early stages of somite formation, at the level of the segmental plate and the epithelial somites, the entire mediolateral extent of the ectoderm overlying the neural tube, the paraxial mesoderm and the lateral plate mesoderm is *Wnt6* positive (Fig. 1A,B). However, during subsequent somite compartmentalization forming the dermomyotome and the sclerotome, *Wnt6* expression disappeared from the medial ectoderm and became restricted to the ectoderm overlying the VLL and the lateral plate mesoderm (Fig. 1A,C,D). This is consistent with earlier observations (Rodriguez-Niedenführ et al., 2003) and indicates that ectodermal Wnt6 is dispensable during the development of the medial somite.

We examined if the loss of *Wnt6* expression in the medial ectoderm is due to an inhibitory action of the axial organs. Axial organs are known to be involved in patterning the medial somite, but so far no patterning influence on the ectoderm has been described (Christ et al., 1992; Dietrich et al., 1997; Marcelle et al., 1997; Münsterberg and Lassar, 1995; Stern and Hauschka, 1995; Stern et al., 1997; Pourquié et al., 1995; Pourquié et al., 1996). To determine the role of axial organs in the regulation of *Wnt6* expression, we separated four to six matured somites and the ectoderm overlying the somites from the neural tube by insertion of an aluminium foil barrier at HH stage 14. After a reincubation period of 12 to 18 hours, we observed an overexpression of *Wnt6* at the level of the barrier, with the expression domain extending into the medial ectoderm (Fig. 2A,D; *n*=8). In a different approach, we microsurgically removed the respective half of neural tube at the same location. Loss of the neural tube also results in a lateromedial expansion of *Wnt6* expression (Fig. 2B,E; *n*=6). This suggests that the lack of *Wnt6* expression in the ectoderm covering the medial region of the matured somites is due to inhibitory signals from the neural tube.

In order to identify the signals from the neural tube which inhibit *Wnt6* expression, we examined the influence of Wnt1 and Wnt3a on *Wnt6* expression. Wnt1 and Wnt3a are expressed in the dorsal neural tube and have been shown to be regulators of medial somite patterning (Münsterberg et al., 1995; Marcelle et al., 1997). After injection of *Wnt1*- or *Wnt3a*-expressing cells into the subectodermal space dorsal to the somites, *Wnt6* expression was totally inhibited at the site of injection (Fig. 2C,F; *n*=12; Wnt1; data not shown). This strongly suggests that the loss of *Wnt6* expression in the medial ectoderm overlying the maturing somites is due to an inhibitory action of Wnt3a and/or Wnt1 from the dorsal neural tube.

Wnt11 in the DML is a transducer of Wnt signals from the neural tube to the ectoderm

We have shown that Wnt1 and Wnt3a are able to inhibit *Wnt6* expression in the medial surface ectoderm. However, *Wnt1* and *Wnt3a* both are expressed in the dorsal neural tube already during early stages of paraxial mesoderm development when *Wnt6* is still expressed in the entire surface ectoderm, including the medial aspect close to the neural tube (Hirsinger et al., 1997; Marcelle et al., 1997; Cauthen et al., 2001). This paradox could be explained by the hypothesis that Wnt1 and Wnt3a do not have a direct effect on *Wnt6* but act via an intermediate signal that is not active before the epithelial somite has developed into dermomyotome and sclerotome.

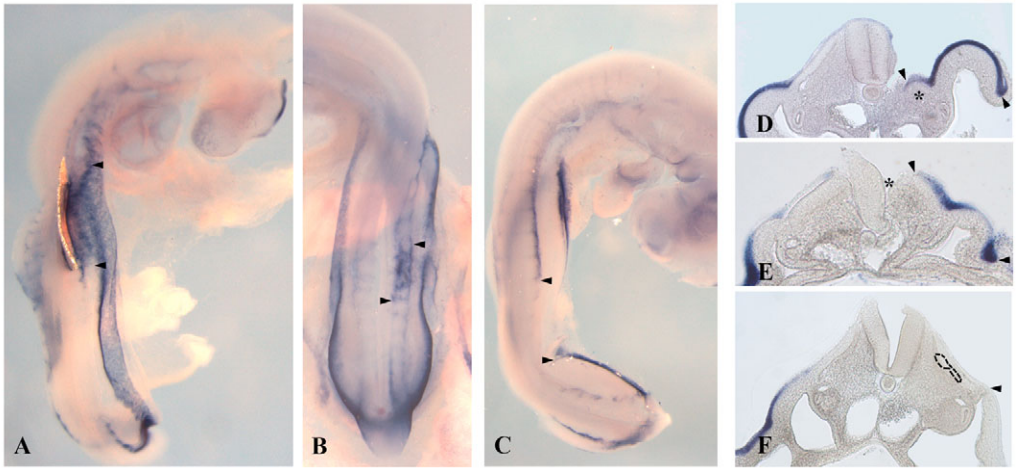

Fig. 2. Wnt signaling from the neural tube downregulates *Wnt6* expression in the ectoderm overlying the somites. (**A**) Separation of the somite from the axial organs by insertion of an aluminium foil barrier induces an upregulation of *Wnt6* expression in the ectoderm covering the somites (between arrowheads), the *Wnt6* expression domain now extending medially to the barrier. (**B**) Removal of the right half of the neural tube induces *Wnt6* expression in the somites on the right (between arrowheads). (**C**) Implantation of Wnt3a-producing cells into the epithelial somites of HH stage 14 embryos leads to the inhibition of *Wnt6* expression in the ectoderm overlying the somites (between arrowheads). (**D**) Transverse section through the operated region in A showing the upregulation of *Wnt6* expression due to the absence of signals from the axial organs. *Wnt6* expression is extended lateromedially (arrowhead). The location of the somite is indicated by an asterisk. (**E**) Transverse section of the embryo shown in B. *Wnt6* expression is extended lateromedially (arrowhead). The asterisk marks the region where the neural tube has been removed. (**F**) Transverse section at the region of operation in C showing the loss of *Wnt6* expression in the ectoderm overlying the somite (arrowhead), position of cells is indicated by broken lines.

The development of the dermomyotome depends on interactions with the overlying ectoderm (reviewed by Scaal and Christ, 2004). To test if dermomyotomal signals influence *Wnt6* expression, we removed the entire dermoymotome, or medial halves of the dermomyotome, and checked for *Wnt6* expression. In both experiments, ectodermal *Wnt6* expression was upregulated at the site of surgery, and expression extended to the medial surface ectoderm, demonstrating that the dermomyotome exerts an inhibitory action on *Wnt6* expression (Fig. 3A-D; *n*=18).

Marcelle and co-workers (Marcelle et al., 1997) have shown that the differentiation of the dorsomedial lip of the dermomyotome (DML) depends on Wnt1 and Wnt3a signaling from the neural tube. To test if the hypothesized intermediate signal, which inhibits *Wnt6* expression in response to Wnts from the neural tube, originates from the DML, we transplanted DMLs from HH13 quail donor embryos to the segmental plate of HH stage 12 chick host embryos. After 12 hours of incubation, *Wnt6* expression in the ectoderm covering the site of DML implantation was downregulated. This shows that the DML has an inhibitory influence on *Wnt6* expression (Fig. 3F; *n*=7).

Wnt11 has been described as a marker gene of the DML, and is expressed in the DML in response to Wnt1 and Wnt3a signaling from the neural tube (Marcelle et al., 1997) (this study see Fig. 3H,I), making it an excellent candidate for the hypothesized intermediate *Wnt6*-inhibitory signal. Indeed, we found that the DML grafts leading to inhibition of Wnt6 in the ectoderm show robust Wnt11 expression (Fig. 3E; *n*=3). We compared the expression pattern of *Wnt11* and *Wnt6* during normal development and found that the onset of dermomyotomal *Wnt11* expression correlates with the downregulation of *Wnt6* in the medial ectoderm (Fig. 1). Together, these data are suggestive of an inhibitory action of Wnt11 on *Wnt6* expression.

To directly test the effect of Wnt11 on *Wnt6* expression, we electroporated *Wnt11* RCAS into prospective dermomyotomal cells of the epithelial somite as described by Scaal et al. (Scaal et al., 2004). To facilitate localization of the construct, we co-electroporated GFP-pCLGFPA, which has no impact on target gene expression (Scaal et al., 2004) (own results not shown). After a reincubation period of 12-16 hours, we observed a robust upregulation of *Wnt11* expression at the site of electroporation (Fig. 4A,B; *n*=5). We analyzed the electroporated embryos for *Wnt6* expression and found that dermomyotomal overexpression of *Wnt11* leads to a total loss of *Wnt6* expression even in the lateral ectoderm (Fig. 4C-E; *n*=7). Conversely, we electroporated a dominant-negative *Wnt11* RCAS-construct, which inhibits endogenous Wnt11 signaling, into the same location. As expected from our previous results, inhibition of Wnt11 signaling lead to a strong upregulation and medial extension of *Wnt6* expression in the ectoderm overlying the electroporated dermomyotome (Fig. 4F-H; *n*=5). Thus, we have shown that Wnt11 is indeed a negative regulator of *Wnt6* expression, and we provide evidence that dermomyotomal Wnt11 transduces the Wnt6-inhibitory signals from the neural tube to the ectoderm.

Wnt11 acts as a somite epithelialization factor

Having found that *Wnt6* expression is restricted to the lateral ectoderm by the inhibitory action of Wnt11 in the medial dermomyotome, we sought to determine the functional significance of this regulatory process.

Wnt6 is known to be an epithelialization factor during somitogenesis (Schmidt et al., 2004; Linker et al., 2005). However, in the matured somites, it is expressed only in the ectoderm overlying the lateralmost margin of the epithelial dermomyotome: the ventrolateral lip (VLL). The epithelialization factor of the medial

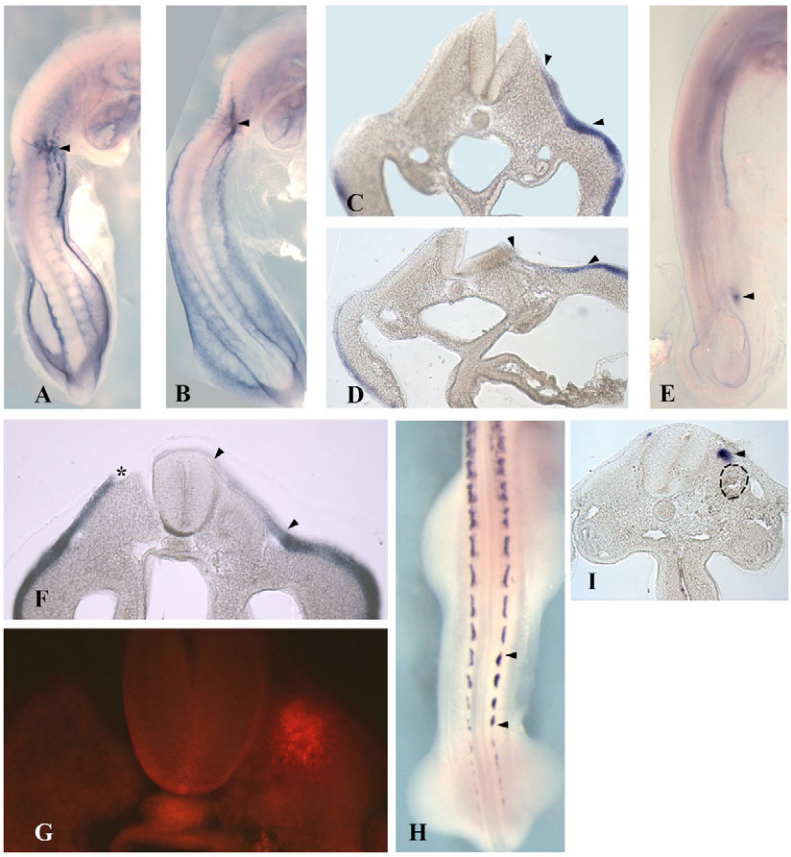

Fig. 3. Signals from the medial dermomyotome downregulate *Wnt6* expression. (**A**) Removal of the whole dermomyotome leads to the upregulation of *Wnt6* expression in the ectoderm overlying the somites (arrowhead). (**B**) Removal of the medial region of the dermomyotome is sufficient to upregulate *Wnt6* expression (arrowhead). (**C**,**D**) Transverse section across the operated region of embryos shown in A,B, respectively, showing a lateromedial extension of ectodermal *Wnt6* expression (medial extent indicated by arrowheads). (**E**) Grafting of a medial dermomyotomal lip to the segmental plate, and in situ hybridization against *Wnt11*. Although no endogenous Wnt11 expression is detectable yet, the graft shows solid *Wnt11* expression (arrowhead). (**F**) Transverse section showing the loss of *Wnt6* expression in the ectoderm covering the paraxial mesoderm after grafting medial dermomytomal lip from quail to the segmental plate (arrowheads; asterisk marks a region on the control side slightly damaged during sectioning). (**G**) Presence of the quail-derived graft shown in F marked by QCPN antibody. (**H**) Implantation of Wnt3a-producing cells into the somites leads to the upregulation of *Wnt11* expression (arrowheads). (**I**) Transverse section at the operated site of the embryo shown in H. *Wnt11* expression is upregulated in the medial dermomyotomal lip (arrowhead); the position of the implanted cells is indicated by a broken line.

dermomyotome is unknown. We hypothesized that after the loss of *Wnt6* expression in the medial ectoderm, Wnt11 in the DML could act as a local epithelialization factor. To investigate this, we tested the expression of markers of the epithelial dermomyotome, *Pax3* and *Paraxis*, after overexpression of *Wnt11* in the somites by electroporation of a Wnt11 RCAS construct. We found an upregulation of *Pax3* and *Paraxis* in the electroporated somites, which showed an abnormal morphology, indicating an excessive epithelialization of the tissue (Fig. 5A-F; n=11). Conversely, *Pax1* expression, which is a marker of the mesenchymal sclerotome, was totally absent (Fig. 5G-I; n=8). These results suggest that Wnt11, like Wnt6, is an epithelialization factor acting on the dermomyotome. By restricting *Wnt6* expression to the ectoderm overlying the VLL, and by *Wnt11* expression restricted to the DML, the persistent epithelial character of the dermomyotome is limited to the marginal lips, which promote ongoing dermomyotomal growth, whereas the central dermomyotomal domains de-epithelialize to form the dorsal dermis (Scaal and Christ, 2004). We, thus, propose an experimental model to explain the maintenance of the dermomyotomal lips in contrast to the early dissociation of the central dermomyotome in the mature somite (Fig. 6).

DISCUSSION

Epithelialization of the paraxial mesoderm

In avian embryos, all cells of the paraxial mesoderm, with the exception of the somitocoele cells, undergo an epithelialization process before they differentiate into definitive tissues. The first epithelial cells appear in the superficial layer of the cranial segmental plate (Christ et al., 1972). Upon signaling from the ectoderm, the bHLH transcription factor *Paraxis* is expressed in these epithelializing cells, together with the tyrosine kinase receptor *EphA4* (Burgess et al., 1996; Sosic et al., 1997; Schmidt et al., 2001). During somite formation, the epithelial cells arrange as spheres to enclose the still mesenchymal somitocoele (Kulesa and Fraser, 2002). Studies of segmental plate explants in culture suggest that the epithelialization step is necessary to synchronize expression of segmentation genes (Maroto et al., 2005). Such a community effect might be a general requirement for coordinated gene expression also in later stages of somite development. Following somite formation, the ventral somite halves de-epithelialize under the influence of signals from the notochord and ventral neural tube (reviewed by Christ et al., 2004). In the dorsal compartment, the somitic epithelium remains intact under the influence of the neural tube and the surface ectoderm, thus forming the dermomyotome (Kenny-Mobbs and Thorogood, 1987; Christ et al., 1992; Spence et al., 1996). Molecular studies have revealed that the formation of the medial dermomyotome depends on Wnt1 and Wnt3a signaling from the dorsal neural tube (Dietrich et al., 1997; Fan et al., 1997; Marcelle et al., 1997; Wagner et al., 2000). Accordingly, in mouse, *Wnt1/3a* knockout mice lose the medial aspect of the dermomyotome (Ikeya and Takada, 1998). The lateral dermomyotome is known to depend on signals from the surface ectoderm (Dietrich et al., 1997; Fan and Tessier-Lavigne, 1994; Fan et al., 1997). In recent studies, Wnt6 has been identified as an epithelialization factor from the surface ectoderm which is required for dermomyotome formation and maintenance (Schmidt et al., 2004; Linker et al., 2005). Wnt6 maintains the epithelial morphology of dermomyotomal cells by promoting *Paraxis* expression via Frizzled7 and β-catenin intracellular signaling (Linker et al., 2005). Until embryonic day 3 the dermomyotome is a continuous epithelial sheet of approximately rectangular shape. At either margin, the epithelial cells form lip-like structures in which cells de-epithelialize and emigrate to form muscle (Gros et al., 2004) and endothelium (Wilting et al., 1995). Later on, the central region of the dermomyotome (CD) deepithelializes completely to give rise to dorsally emigrating dermal and subcutaneous precursor cells, and ventrally emigrating proliferative muscle progenitor cells and satellite cells (Gros et al., 2005; Relaix et al., 2005; Ben-Yair and Kalcheim, 2005). By contrast, the DML and VLL persist as two separate epithelial proliferation zones which are required for ongoing mediolateral growth of the dermomyotome and its derivatives. At embryonic day 7, when the entire dermomyotome has developed into definite tissues, the DML and VLL disintegrate (Ordahl et al., 2001; Venters and Ordahl, 2002). The molecular basis for the differential timing of dermomyotomal de-epithelialization in the margins and the CD has remained elusive.

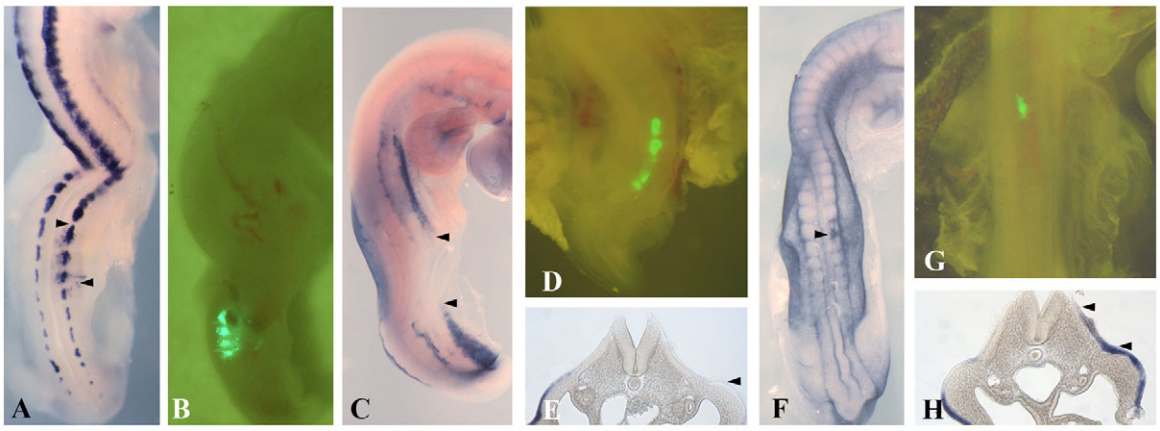

Fig. 4. Inhibitory effect of Wnt11 on the expression of ectodermal *Wnt6*. (**A**) Electroporation of Wnt11 RCAS constructs along with GFP plasmids leads to the upregulation of *Wnt11* expression in somites (arrowheads). (**B**) Fluorescence image of the embryo in A showing the localization of the constructs electroporated. (**C**) Overexpression of Wnt11 leads to the loss of *Wnt6* expression in the ectoderm (arrowheads). (**D**) Embryo in C viewed under fluorescence light showing the position of the electroporated Wnt11 construct. (**E**) Transverse section of the operated region of embryo in C, showing the loss of *Wnt6* expression in the ectoderm (arrowhead). (**F**) Overexpression of dnWnt11 results in the upregulation of *Wnt6* expression in the ectoderm (arrowhead). (**G**) Localization of dnWnt11 plasmid shown by fluorescence emitted by the GFP plasmids that were co-electroporated. (**H**) Transverse section of the embryo in F at the operated site, showing a lateromedial extension of *Wnt6* expression (arrowheads).

Wnt1/3a signaling from the neural tube regulates ectodermal *Wnt6* expression via dermomyotomal Wnt11

In this study, we present functional data to explain the molecular regulation of the de-epithelialization dynamics of the mature dermomyotome. We show that the expression of *Wnt6*, which keeps the dermomyotome in an epithelial state (Schmidt et al., 2004; Linker et al., 2005), is downregulated in the medial and central paraxial ectoderm by signals from the neural tube. To extend earlier findings that Wnt1 and Wnt3a from the dorsal neural tube pattern the medial dermomyotome (Dietrich et al., 1997; Fan et al., 1997; Marcelle et al., 1997; Wagner et al., 2000), we show here that these signals also pattern ectodermal gene expression and inhibit *Wnt6* expression in the ectoderm overlying the medial and central dermomyotome. Further upstream in this signaling cascade, Wnt1/3a signaling depends on BMP4 activity in the neural tube (Marcelle et al., 1997). In confirmation of our results, we observed that inhibition of BMP4 by overexpression of Noggin in the neural tube also leads to an extension of *Wnt6* expression to the medial paraxial ectoderm (data not shown). However, as *Wnt1/3a* are already expressed in early stages of paraxial mesoderm development, while *Wnt6* is still expressed throughout the surface ectoderm, we searched for an intermediate regulator restricting Wnt1/3a action on the somite stages following dermomyotome formation. We found that the inhibition of medial *Wnt6* expression depends on the presence of the DML, thus identifying a patterning influence of the paraxial mesoderm on the overlying ectoderm. Marcelle and co-workers (Marcelle et al., 1997) have shown that the DML is demarcated by *Wnt11* expression, and that Wnt11 expression depends on Wnt1/3a signaling from the neural tube. We therefore tested if Wnt11 is able to inhibit ectodermal *Wnt6* expression, and performed localized overexpression of Wnt11 in the dorsal somites. We found that indeed dermomyotomal Wnt11 inhibits *Wnt6* expression in the overlying ectoderm, making it an excellent candidate for the intermediate factor restricting the *Wnt6* inhibitory action of Wnt1/3a to later stages of somite development.

Wnt11 is an epithelializing factor maintaining the DML

Thus, in the mature dermomyotome, the epithelializing impact of Wnt6 is restricted to the VLL, whereas the CD is devoid of epithelializing signals and becomes mesenchymal to form muscle

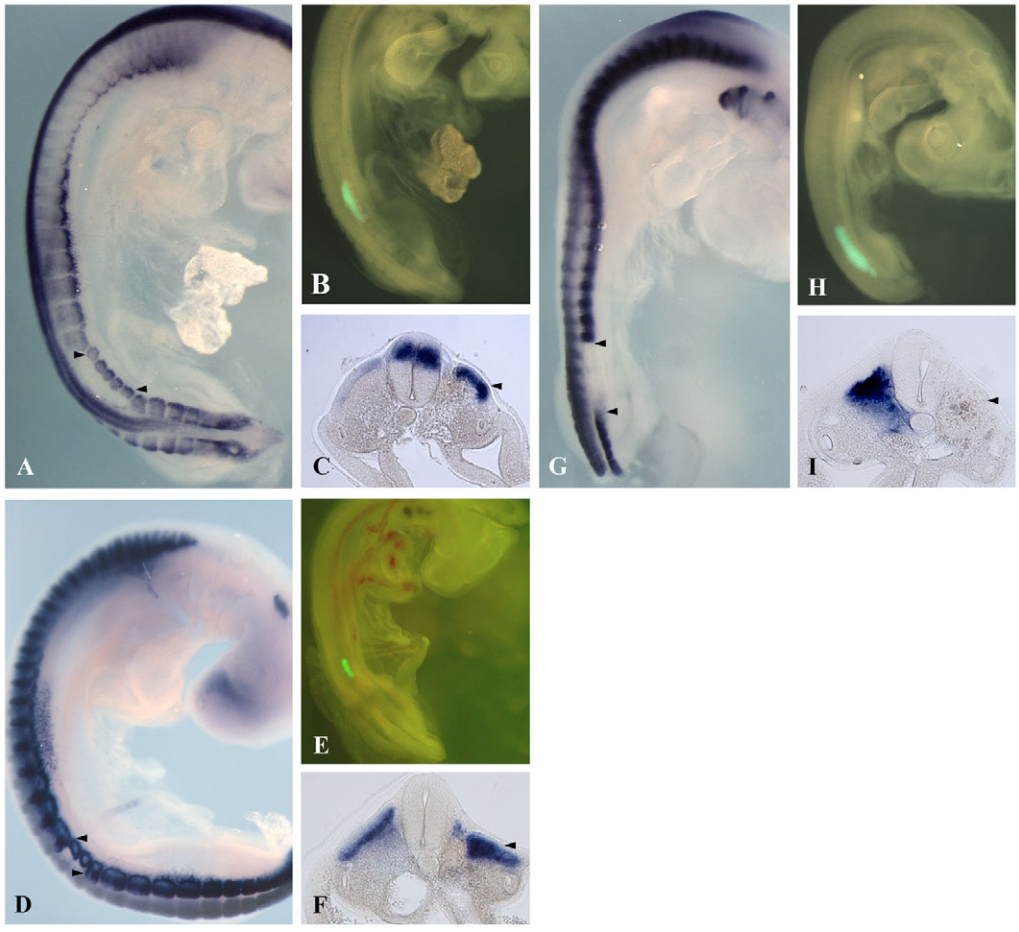

Fig. 5. Function of Wnt11 in the epithelialization of somites. (**A**) Overexpression of Wnt11 RCAS by electroporation leads to small, epithelialized somites showing strong expression of *Pax3* (arrowheads). (**B**) Fluorescence image of the embryo in A, showing the localization of the constructs electroporated. (**C**) Transverse section of the operated region in A, showing the upregulation of *Pax3* expression (arrowhead) and the epithelialized morphology of the somite. (**D**) Overexpression of *Wnt11* leads to small, epithelialized somites showing strong expression of *Paraxis* (arrowheads). (**E**) Fluorescence image of the embryo in D, showing the localization of the constructs electroporated. (**F**) Transverse section of the operated region of embryo in D, showing the upregulation of *Paraxis* expression (arrowhead). (**G**) Overexpression of *Wnt11* leads to the complete loss of *Pax1* expression (arrowheads). (**H**) Localization of the Wnt11 construct in the embryo shown in G. (**I**) Transverse section at the level of operation of the embryo in (G) showing loss of *Pax-1* expression (arrowhead). The epithelial structure of the somite is maintained.

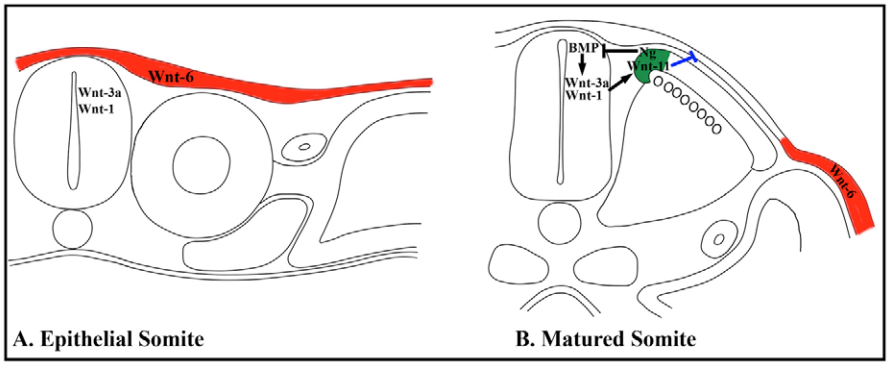

Fig. 6. A model for the regulation of ectodermal *Wnt6* expression and dermomyotome epithelialization. Arrows indicate positive inductions, while a line with bars represents inhibitory actions. (**A**) Epithelial somite: the expression of *Wnt6* (red) is observed in the whole ectoderm covering the neural tube, paraxial mesoderm and the LPM. (**B**) Compartmentalized somite: *Wnt6* expression (red) is restricted to the ectoderm covering the VLL and the LPM. BMP induces *Wnt3a* and *Wnt1* expression in the neural tube, which in turn induces *Wnt11* (green) expression in the DML. *Wnt11* expression takes over the function of maintaining epithelialization in the DML and downregulates *Wnt6* in the ectoderm overlying the central and medial region of the dermomyotome. Thus, epithelial morphology is restricted to the DML and VLL by dermomyotomal Wnt11 and ectodermal Wnt6, respectively.

and connective tissue (Gros et al., 2005; Ben-Yair and Kalcheim, 2005). In addition, the cranial and caudal dermomyotomal lips, which give rise to myotomal cells and persist longer than the CD (Ordahl et al., 2001; Gros et al., 2004), are also exposed to prolonged Wnt6 activity as *Wnt6* expression is maintained in the ectodermal ridges extending into the intersegmental clefts between the adjacent somites until embryonic day 4 (Rodriguez-Niedenführ et al., 2003) (our observation). How, then, is the DML kept epithelial? We found that overexpression of *Wnt11* leads to an overexpression of the epithelial markers *Pax3* and *Paraxis*, whereas the marker of sclerotomal fate, *Pax1*, is absent. Moreover, the affected somites displayed an abnormal morphology and reduced size, indicating an excessive epithelialization including ventral somitic cells (Schmidt et al., 2004), thus preventing sclerotome formation. Thus, we could show that Wnt11 is a mesoderm-intrinsic epithelialization factor that, upon induction from the neural tube, maintains the epithelial state of the DML while restricting *Wnt6* expression to the ectoderm overlying the VLL. In the literature, Wnt11 has been described to be involved in convergence extension movements during zebrafish gastrulation (Heisenberg et al., 2000). Here, we present a novel dual role of Wnt11 in downregulation of ectodermal *Wnt6* expression and maintenance of the epithelial state of the DML. It remains to be elucidated if Wnt11 is furthermore required for the cell movements during myotomal cell recruitment in the DML. An important aspect of our results is that Wnt11 represents the first known mesodermal epithelialization factor acting on its cells of origin in a paracrine fashion, thus establishing a fundamental difference between the regulation of epithelialization in the DML and the other dermomyotomal regions, which are thought to depend entirely on ectodermal signals.

A model on the regulation of the dermomyotomal epithelium

Taken together, our data lead us to propose a conclusive model explaining both the dynamic expression pattern of *Wnt6* and the regulation of the epithelial morphology of the dermomyotome (Fig. 6). Prior to somite compartmentalization, *Wnt6* is expressed in the entire surface ectoderm and promotes epithelialization of the early somites via β-catenin and Paraxis (Schmidt et al., 2004; Linker et al., 2005). Following dermomyotome formation, Wnt1 and Wnt3a, which are secreted by the dorsal neural tube upon local BMP signaling, induce *Wnt11* expression in the DML (Marcelle et al., 1997). Wnt11 inhibits *Wnt6* expression in the ectoderm overlying the medial and central dermomyotome, thus restricting *Wnt6* expression to the lateral ectoderm overlying the VLL. As both, Wnt6 and Wnt11, are epithelialization factors, the DML (via Wnt11) and the VLL (via Wnt6) remain epithelial and contribute to dermomyotomal growth, whereas cells in the CD de-epithelialize to differentiate. Importantly, the intermediate activity of Wnt11 as transducer of neural tube-derived signaling provides a timing mechanism restricting this process to the fully formed dermomyotome.

We thank Dr Christophe Marcelle for providing the Wnt11 probe, Dr Philippa Francis-West for Wnt11 RCAS and dnWnt11 RCAS constructs, Dr Andreas Kispert for the Wnt3a- and Wnt1-expressing cells, and Dr Richard Harland for CHO cells expressing noggin. We thank Dr Marc Rodriguez-Niedenführ for his excellent suggestions and help provided during this study. We also thank Ulrike Pein for excellent technical assistance. This study was supported by the Deutsche Forschungsgemeinschaft (SFB-592, A1 to B.C. and M.S.) and the European Network of Excellence, MYORES (to B.C. and M.S.).

References

Anakwe, K., Robson, L., Hadley, J., Buxton, P., Church, V., Allen, S., Hartmann, C., Harfe, B., Nohno, T., Brown, A. M. et al. (2003). Wnt signalling regulates myogenic differentiation in the developing avian wing. *Development* **130**, 3503-3514.

Aoyama, H. and Asamoto, K. (1988). Determination of somite cells: independence of cell differentiation and morphogenesis. *Development* **104**, 15-28.

Ben-Yair, R. and Kalcheim, C. (2005). Lineage analysis of the avian dermomyotome sheet reveals the existence of single cells with both dermal and muscle progenitor fates. *Development* **132**, 689-701.

Brand-Saberi, B., Ebensperger, C., Wilting, J., Balling, R. and Christ, B. (1993). The ventralizing effect of the notochord on somite differentiation in chick embryos. *Anat. Embryol.* **188**, 239-245.

Burgess, R., Rawls, A., Brown, D., Bradley, A. and Olson, E. N. (1996). Requirement of the paraxis gene for somite formation and musculoskeletal patterning. *Nature* **384**, 570-573.

Cadigan, K. M. and Nusse, R. (1997). Wnt signaling: a common theme in animal development. *Genes Dev.* **11**, 3286-3305.

Cauthen, C. A., Berdougo, E., Sandler, J. and Burrus, L. W. (2001). Comparative analysis of the expression patterns of Wnts and Frizzleds during early myogenesis in chick embryos. *Mech. Dev.* **104**, 133-138.

Chiang, C., Litingtung, Y., Lee, E., Young, K. E., Corden, J. L., Westphal, H. and Beachy, P. A. (1996). Cyclopia and defective axial patterning in mice lacking Sonic hedgehog gene function. *Nature* **383**, 407-413.

Christ, B., Jacob, H. J. and Jacob, M. (1972). [Experimental analysis of somitogenesis in the chick embryo]. *Z. Anat. Entwicklungsgesch.* **138**, 82-97.
Christ, B., Brand-Saberi, B., Grim, M. and Wilting, J. (1992). Local signalling in dermomyotomal cell type specification. *Anat. Embryol.* **186**, 505-510.
Christ, B., Huang, R. and Scaal, M. (2004). Formation and differentiation of the avian sclerotome. *Anat. Embryol.* **208**, 333-350.
Dickinson, M. E. and McMahon, A. P. (1992). The role of Wnt genes in vertebrate development. *Curr. Opin. Genet. Dev.* **2**, 562-566.
Dietrich, S., Schubert, F. R. and Lumsden, A. (1997). Control of dorsoventral pattern in the chick paraxial mesoderm. *Development* **124**, 3895-3908.
Fan, C. M. and Tessier-Lavigne, M. (1994). Patterning of mammalian somites by surface ectoderm and notochord: evidence for sclerotome induction by a hedgehog homolog. *Cell* **79**, 1175-1186.
Fan, C. M., Lee, C. S. and Tessier-Lavigne, M. (1997). A role for WNT proteins in induction of dermomyotome. *Dev. Biol.* **191**, 160-165.
Gros, J., Scaal, M. and Marcelle, C. (2004). A two-step mechanism for myotome formation in chick. *Dev. Cell* **6**, 875-882.
Gros, J., Manceau, M., Thome, V. and Marcelle, C. (2005). A common somitic origin for embryonic muscle progenitors and satellite cells. *Nature* **435**, 954-958.
Gumbiner, B. M. (1996). Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell* **84**, 345-357.
Hamburger, V. and Hamilton, H. L. (1951). A series of normal stages in the development of chick embryo. *J. Morphol.* **88**, 49-92.
Heisenberg, C. P., Tada, M., Rauch, G. J., Saude, L., Concha, M. L., Geisler, R., Stemple, D. L., Smith, J. C. and Wilson, S. W. (2000). Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* **405**, 76-81.
Hinck, L., Nathke, I. S., Papkoff, J. and Nelson, W. J. (1994). Beta-catenin: a common target for the regulation of cell adhesion by Wnt-1 and Src signaling pathways. *Trends Biochem. Sci.* **19**, 538-542.
Hirsinger, E., Duprez, D., Jouve, C., Malapert, P., Cooke, J. and Pourquié, O. (1997). Noggin acts downstream of Wnt and Sonic Hedgehog to antagonize BMP4 in avian somite patterning. *Development* **124**, 4605-4614.
Huelsken, J. and Birchmeier, W. (2001). New aspects of Wnt signaling pathways in higher vertebrates. *Curr. Opin. Genet. Dev.* **11**, 547-553.
Ikeya, M. and Takada, S. (1998). Wnt signaling from the dorsal neural tube is required for the formation of the medial dermomyotome. *Development* **125**, 4969-4976.
Itaranta, P., Lin, Y., Perasaari, J., Roel, G., Destree, O. and Vainio, S. (2002). Wnt-6 is expressed in the ureter bud and induces kidney tubule development in vitro. *Genesis* **32**, 259-268.
Janson, K., Cohen, E. D. and Wilder, E. L. (2001). Expression of DWnt6, DWnt10, and DFz4 during Drosophila development. *Mech. Dev.* **103**, 117-120.
Johnson, R. L., Laufer, E., Riddle, R. D. and Tabin, C. (1994). Ectopic expression of Sonic hedgehog alters dorsal-ventral patterning of somites. *Cell* **79**, 1165-1173.
Kenny-Mobbs, T. and Thorogood, P. (1987). Autonomy of differentiation in avian branchial somites and the influence of adjacent tissues. *Development* **100**, 449-462.
Kos, L., Chiang, C. and Mahon, K. A. (1998). Mediolateral patterning of somites: multiple axial signals, including Sonic hedgehog, regulate Nkx-3.1 expression. *Mech. Dev.* **70**, 25-34.
Kulesa, P. M. and Fraser, S. E. (2002). Cell dynamics during somite boundary formation revealed by time-lapse analysis. *Science* **298**, 991-995.
Kuratani, S., Martin, J. F., Wawersik, S., Lilly, B., Eichele, G. and Olson, E. N. (1994). The expression pattern of the chick homeobox gene gMHox suggests a role in patterning of the limbs and face and in compartmentalization of somites. *Dev. Biol.* **161**, 357-369.
Lamb, T. M., Knecht, A. K., Smith, W. C., Stachel, S. E., Economides, A. N., Stahl, N., Yancopolous, G. D. and Harland, R. M. (1993). Neural induction by the secreted polypeptide noggin. *Science* **262**, 713-718.
Linker, C., Lesbros, C., Gros, J., Burrus, L. W., Rawls, A. and Marcelle, C. (2005). beta-Catenin-dependent Wnt signalling controls the epithelial organisation of somites through the activation of paraxis. *Development* **132**, 3895-3905.
Loganathan, P. G., Nimmagadda, S., Huang, R., Scaal, M. and Christ, B. (2005). Comparative analysis of the expression patterns of Wnts during chick limb development. *Histochem. Cell Biol.* **123**, 195-201.
Marcelle, C., Stark, M. R. and Bronner-Fraser, M. (1997). Coordinate actions of BMPs, Wnts, Shh and noggin mediate patterning of the dorsal somite. *Development* **124**, 3955-3963.
Maroto, M., Dale, J. K., Dequeant, M. L., Petit, A. C. and Pourquié, O. (2005). Synchronised cycling gene oscillations in presomitic mesoderm cells require cell-cell contact. *Int. J. Dev. Biol.* **49**, 309-315.
Münsterberg, A. E. and Lassar, A. B. (1995). Combinatorial signals from the neural tube, floor plate and notochord induce myogenic bHLH gene expression in the somite. *Development* **121**, 651-660.
Münsterberg, A. E., Kitajewski, J., Bumcrot, D. A., McMahon, A. P. and Lassar, A. B. (1995). Combinatorial signaling by Sonic hedgehog and Wnt family members induces myogenic bHLH gene expression in the somite. *Genes Dev.* **9**, 2911-2922.
Nieto, M. A., Patel, K. and Wilkinson, D. G. (1996). In situ hybridization analysis of chick embryos in whole mount and tissue sections. *Methods Cell Biol.* **51**, 219-235.
Ordahl, C. P. and Le Douarin, N. M. (1992). Two myogenic lineages within the developing somite. *Development* **114**, 339-353.
Ordahl, C. P., Berdougo, E., Venters, S. J. and 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.
Parr, B. A. and McMahon, A. P. (1994). Wnt genes and vertebrate development. *Curr. Opin. Genet. Dev.* **4**, 523-528.
Pourquié, O., Coltey, M., Teillet, M. A., Ordahl, C. and Le Douarin, N. M. (1993). Control of dorsoventral patterning of somitic derivatives by notochord and floor plate. *Proc. Natl. Acad. Sci. USA* **90**, 5242-5246.
Pourquié, O., Coltey, M., Breant, C. and Le Douarin, N. M. (1995). Control of somite patterning by signals from the lateral plate. *Proc. Natl. Acad. Sci. USA* **92**, 3219-3223.
Pourquié, O., Fan, C. M., Coltey, M., Hirsinger, E., Watanabe, Y., Breant, C., Francis-West, P., Brickell, P., Tessier-Lavigne, M. and Le Douarin, N. M. (1996). Lateral and axial signals involved in avian somite patterning: a role for BMP4. *Cell* **84**, 461-471.
Relaix, F., Rocancourt, D., Mansouri, A. and Buckingham, M. (2005). A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. *Nature* **435**, 948-953.
Rodriguez-Niedenführ, M., Dathe, V., Jacob, H. J., Prols, F. and Christ, B. (2003). Spatial and temporal pattern of Wnt-6 expression during chick development. *Anat. Embryol.* **206**, 447-451.
Scaal, M. and Christ, B. (2004). Formation and differentiation of the avian dermomyotome. *Anat. Embryol.* **208**, 411-424.
Scaal, M., Gros, J., Lesbros, C. and Marcelle, C. (2004). In ovo electroporation of avian somites. *Dev. Dyn.* **229**, 643-650.
Schmidt, C., Christ, B., Maden, M., Brand-Saberi, B. and Patel, K. (2001). Regulation of EphA4 expression in paraxial and lateral plate mesoderm by ectoderm-derived signals. *Dev. Dyn.* **220**, 377-386.
Schmidt, C., Stoeckelhuber, M., McKinnell, I., Putz, R., Christ, B. and Patel, K. (2004). Wnt 6 regulates the epithelialisation process of the segmental plate mesoderm leading to somite formation. *Dev. Biol.* **271**, 198-209.
Schubert, F. R., Mootoosamy, R. C., Walters, E. H., Graham, A., Tumiotto, L., Munsterberg, A. E., Lumsden, A. and Dietrich, S. (2002). Wnt6 marks sites of epithelial transformations in the chick embryo. *Mech. Dev.* **114**, 143-148.
Schubert, M., Holland, L. Z., Stokes, M. D. and Holland, N. D. (2001). Three amphioxus Wnt genes (AmphiWnt3, AmphiWnt5, and AmphiWnt6) associated with the tail bud: the evolution of somitogenesis in chordates. *Dev. Biol.* **240**, 262-273.
Sosic, D., Brand-Saberi, B., Schmidt, C., Christ, B. and Olson, E. N. (1997). Regulation of paraxis expression and somite formation by ectoderm- and neural tube-derived signals. *Dev. Biol.* **185**, 229-243.
Spence, M. S., Yip, J. and Erickson, C. A. (1996). The dorsal neural tube organizes the dermamyotome and induces axial myocytes in the avian embryo. *Development* **122**, 231-241.
Stern, H. M. and Hauschka, S. D. (1995). Neural tube and notochord promote in vitro myogenesis in single somite explants. *Dev. Biol.* **167**, 87-103.
Stern, H. M., Lin-Jones, J. and Hauschka, S. D. (1997). Synergistic interactions between bFGF and a TGF-beta family member may mediate myogenic signals from the neural tube. *Development* **124**, 3511-3523.
Tao, Q., Yokota, C., Puck, H., Kofron, M., Birsoy, B., Yan, D., Asashima, M., Wylie, C. C., Lin, X. and Heasman, J. (2005). Maternal wnt11 activates the canonical wnt signaling pathway required for axis formation in Xenopus embryos. *Cell* **120**, 736-737.
Venters, S. J. and Ordahl, C. P. (2002). Persistent myogenic capacity of the dermomyotome dorsomedial lip and restriction of myogenic competence. *Development* **129**, 3873-3885.
Wagner, J., Schmidt, C., Nikowits, W., Jr and Christ, B. (2000). Compartmentalization of the somite and myogenesis in chick embryos are influenced by wnt expression. *Dev. Biol.* **228**, 86-94.
Wilting, J., Brand-Saberi, B., Huang, R., Zhi, Q., Kontges, G., Ordahl, C. P. and Christ, B. (1995). Angiogenic potential of the avian somite. *Dev. Dyn.* **202**, 165-171.