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# Severe defects in the formation of epaxial musculature in *open brain* (*opb*) mutant mouse embryos

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## SUMMARY

**The differentiation of somite derivatives is dependent on signals from neighboring axial structures. While ventral signals have been described extensively, little is known about dorsal influences, especially those from the dorsal half of the neural tube. Here, we describe severe phenotypic alterations in dorsal somite derivatives of homozygous *open brain* (*opb*) mutant mouse embryos which suggest crucial interactions between dorsal neural tube and dorsal somite regions. At Theiler stage 17 (day 10.5 post coitum) of development, strongly altered expression patterns of *Pax3* and *Myf5* were observed in dorsal somite regions indicating that the dorsal myotome and dermomyotome were not differentiating properly. These abnormalities were later followed by the absence of epaxial (dorsal) musculature; whereas, body wall and limb musculature formed normally. Analysis of *Mox1* and *Pax1* expression in *opb* embryos revealed additional defects in the differentiation of the dorsal sclerotome. The observed abnormalities coincided with defects in differentiation of dorsal neural tube regions. The implications of our findings for interactions between dorsal neural tube, surface ectoderm and dorsomedial somite regions in specifying epaxial musculature are discussed.**

Key words: neural tube, *Pax* genes, myogenic factors, somite, hypaxial muscle, skeleton, mouse

## INTRODUCTION

In the vertebrate body, the axial skeleton, all skeletal muscles and the dermis of the back are derived from segmentally organized mesoderm structures, the somites. During embryonic development, somites are generated lateral to the developing neural tube from the unsegemented paraxial mesoderm by the formation of epithelial spheres (reviewed by Christ and Ordahl, 1995; Keynes and Stern, 1988; Rong et al., 1992). Shortly after segmentation, the early ventral somite epithelium and somitocoel cells form the mesenchymal sclerotome, from which the various elements of the axial skeleton and many connective tissues are formed (Bagnall et al., 1989; Christ and Ordahl, 1995; Christ and Wilting, 1992). The early dorsal somite epithelium remains epithelial as the dermomyotome and generates, as a second epithelial layer, the myotome, from which the skeletal muscles later develop. The remaining dermomyotome then differentiates into dermis and additional muscle populations (Christ and Ordahl, 1995).

Several laboratories have demonstrated that the original compartmentalization of the early somites into dermomyotome and sclerotome is dependent on signals from neighboring structures: notochord and neural tube. It has been shown that notochord or floor plate can influence somitic cells to differentiate as sclerotome derivatives and to express the first molecular marker of the sclerotome, *Pax1* (reviewed by Christ and Ordahl, 1995). When notochord or floor plate is implanted next to dorsal somitic regions, these regions no longer express *Pax3*, but activate *Pax1*. Subsequently, they fail to form myotome but do differentiate into cartilage anlagen instead of muscle and dermis (Bober et al., 1994; Brand-Saberi et al., 1993; Goulding et al., 1994; Pourquié et al., 1993). Recently, the *Sonic hedgehog* gene product has been recognized as a crucial ventral signal required for sclerotome specification (Fan and Tessier-Lavigne, 1994; Johnson et al., 1994).

Much less is known about signals involved in dorsal somite specification. Tissue culture explants have demonstrated that differentiation of somites into a dermomyotome is not a default pathway but rather depends on neighboring axial structures (Buffinger and Stockdale, 1994, 1995; Münsterberg and Lassar, 1995; Stern and Hauschka, 1995). Initially, *Pax3* is expressed throughout the presegmental plate and becomes restricted to dorsal regions when the epithelial somites form. In the differentiating somite, *Pax3* is restricted to dermomyotomal cells (Bober et al., 1994; Goulding et al., 1994; Williams and Ordahl, 1994) and the myogenic factors *Myf5*, *myogenin*, and *Myod1* are expressed in the myotome (Smith et al., 1994; reviewed by Buckingham, 1994; Olson and Klein, 1994). Incubation of presegmental plate mesoderm, or newly formed somites, in vitro with neural tube explants results in the

maintenance of *Pax3* and the induction of myogenic marker genes in paraxial mesoderm (Buffinger and Stockdale, 1994, 1995; Fan and Tessier-Lavigne, 1994; Münsterberg and Lassar, 1995; Stern and Hauschka, 1995). In addition, surface ectoderm has been shown to maintain the expression of *Pax3* and to induce *Pax7* in paraxial mesoderm (Fan and Tessier-Lavigne, 1994; Johnson et al., 1994).

From the dermomyotome, two different myogenic lineages are generated (Ordahl and Le Douarin, 1992; see also Fig. 2C). Myogenic cells derived from the dorsomedial dermomyotome differentiate into epaxial (dorsal) musculature; whereas, myogenic cells generated from the dorsolateral dermomyotome form hypaxial (ventral) musculature. From experiments in chicken embryos, it has been suggested that interactions between dorsal somite and neural tube are crucial for differentiation of the dorsal myotome (Bober et al., 1994; Christ et al., 1992; Rong et al., 1992; Teillet and LeDouarin, 1983). At present, the nature and the source of the neural tube signals are not known.

We have recently discovered a new mouse mutant (*open brain*; *opb*) which exhibits severe defects in neural tube closure and in the development of the spinal cord, spinal ganglia, the axial skeleton, limbs and eyes (Günther et al., 1994). In homozygous *opb* mutant embryos, the spinal cord protrudes from the back of the embryo and in small sections within the abnormal spinal cord, the expression of genes normally active in dorsal cells is completely lost. These abnormalities suggest a defect in the specification or differentiation of dorsal-most regions in the developing spinal cord.

To investigate whether the dorsal neural tube abnormalities observed in *opb* embryos are associated with defects in developing somites, we studied the maturation of somites and their derivatives. In this study, we describe developmental defects in dorsal somite and neural tube regions in *opb* mutant embryos. The abnormalities observed early in the differentiation of dorsal dermomyotome and myotome regions were followed by the loss of epaxial musculature; whereas, hypaxial muscle was formed normally. Thus, the defects in *opb* mutant embryos suggest that during normal development, crucial dorsal signals are required for the proper specification of dorsal neural tube and dorsal somite regions.

## MATERIALS AND METHODS

### Maintenance, production and staging of *opb* mutants

The origin and breeding of *opb* mutant mice and the generation of homozygous mutant embryos have been described previously (Günther et al., 1994). Staging of mouse embryos was performed as Theiler stages (TS), described by Kaufman (1992). *opb* mutant embryos were phenotypically identified by their exencephaly, their abnormal gene expression patterns, and/or the pronounced protrusion of the spinal cord. At TS15, *opb* mutant embryos could either be identified by their exencephaly, or in embryos where the neural tube was closed, by the abnormal shape of the developing forebrain region: *opb* mutant embryos did not exhibit the dorsal constriction in the roof of the diencephalon, which was clearly visible in wild-type embryos at this developmental stage.

### In situ hybridization

In situ hybridizations of sections and whole mount embryos were carried out as described by Günther et al. (1994). For *Pax3* in situ hybridizations, a 520 nucleotide (Nu) riboprobe (Goulding et al., 1991), for *Wnt3a,* a 750 Nu riboprobe (Roelink and Nusse, 1991); for *Myf5,* a 310 Nu riboprobe (Ott et al., 1991); for *myogenin,* a 1 kb riboprobe (Edmondson and Olson, 1989); for *Mox1,* a 515 Nu riboprobe (Candia et al., 1992); for *Myod1,* a 1.7 kb riboprobe (Davis et al., 1987); and for *Pax1,* a 1.0 kb probe (for whole-mount in situ hybridizations) or a 313 Nu probe (for in situ hybridization of sections; A. Neubüser and R. Balling, personal communication; Deutsch et al., 1988) was generated. It should be noted that both *Pax1* probes contained the paired box region and exhibited some cross-hybridization to regions of strong *Pax9* gene expression (A. Neubüser and R. Balling, personal communication).

## RESULTS

### Defects in dorsal myotome and dermomyotome regions of *opb* mutant embryos

At Theiler stage 17 (TS17; day 10.5 post coitum; p.c.) of mouse development, somites are differentiated into dermomyotome, myotome and sclerotome (compare with Christ and Ordahl, 1995). The formation of these derivatives can be examined by studying the specific patterns of several developmentally expressed genes. *Myf5* expression in wild-type embryos is found in both dorsal and ventral myotome regions including the somitic bud (Figs 1A,C, 2). Weak *Pax3* expression can be seen in both the dorsal and ventral dermomyotome and strongly in the elongating somitic bud (Figs 1E, 2). It should be noted that during normal somite development the relative topographical positions of derivatives of the early dorsal somite regions change in such a way that derivatives of early dorsomedial regions will form the dorsal myotome and dermomyotome, and early dorsolateral regions will generate ventral myotome and dermomyotome regions (see Fig. 2 for details).

In *opb* mutant embryos, however, analysis of *Myf5* and *Pax3* gene expression revealed a drastically altered expression pattern in dorsal regions of the developing myotome and dermomyotome (Fig. 1B,D,F). In cervical regions, the cap-like dorsal-most expression of *Myf5*, observed in normal embryos, was missing in *opb* embryos (Fig. 1B,D, small arrowhead). In lower cervical and upper thoracic regions (from approximately somite 12 (7th cervical) to somite 17 (5th thoracic)), the expression of *Myf5* was strongly reduced in the anterodorsal and posterodorsal regions of each somite resulting in a flame-like appearance of the remaining dorsal myotome (Fig. 1D, arrowheads). Most drastically, in lower thoracic and lumbar axial regions almost no expression of *Myf5* could be detected in dorsal myotome regions (Fig. 1D, arrows). In more caudal regions, the dorsal *Myf5* pattern appeared normal. Expression of *Myf5* in ventral myotome regions, including the somitic bud, appeared normal, except for minor changes in the shape of the somitic bud and diffuse segment boundaries (Fig. 1B,D). Also, expression of *Pax3* in the dorsal dermomyotome of TS17 *opb* mutant embryos was drastically reduced in lower thoracic regions (Fig. 1F, arrows) and exhibited a flame-like pattern in lower cervical and upper thoracic regions, very similar to the aberrant *Myf5* pattern (Fig. 1F, arrowheads). *Pax3* expression patterns in ventral dermomyotome regions and the somitic bud appeared relatively normal (Fig. 1F). These results suggested that, in *opb* mutant embryos, the ventral myotome and ventral

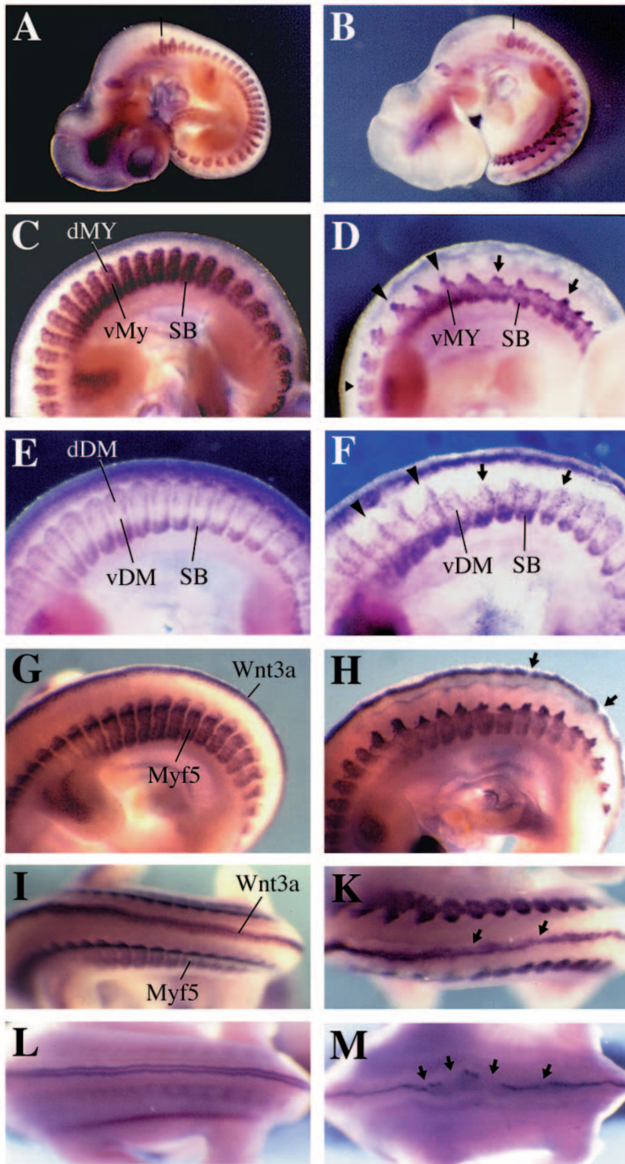


**Fig. 1.** Expression of *Myf5*, *Pax3* and *Wnt3a* in TS17 and TS21 wild-type and *opb* mutant embryos after whole-mount in situ hybridization. (A,C) Lateral views of wild-type and (B,D) *opb* mutant embryos after hybridization to *Myf5*. The first cervical somite (6th somite) is indicated in A and B. (E,F) Lateral views of wild-type and *opb* mutant embryos after hybridization to *Pax3*, respectively. (G,H) Lateral and (I,K) dorsal views of wild-type (G,I) and *opb* mutant (H,K) embryos after hybridization to *Myf5* and *Wnt3a*. (L,M) Dorsal views of TS21 wild-type and *opb* mutant embryos after hybridization to *Wnt3a*, respectively. In *opb* mutant embryos, the formation of dorsal myotome, assayed by *Myf5* gene expression (arrows and arrowheads in D), and dorsal dermomyotome, assayed by *Pax3* expression (arrows and arrowheads in F), is severely affected, whereas the ventral myotome and ventral dermomyotome forms almost normally. The defect in dorsal myotome regions appeared in the same axial regions where defects in the neural tube were observed. Note the patchy appearance of the dorsal band of *Wnt3a*-positive cells at TS17 (arrows in H,K) in regions where abnormal *Myf5* expression was also evident, and the gaps in the dorsal band of *Wnt3a* at TS21 (arrows in M). dDM, dorsal dermomyotome; dMY, dorsal myotome; SB, somitic bud; vDM, ventral dermomyotome; vMY, ventral myotome.

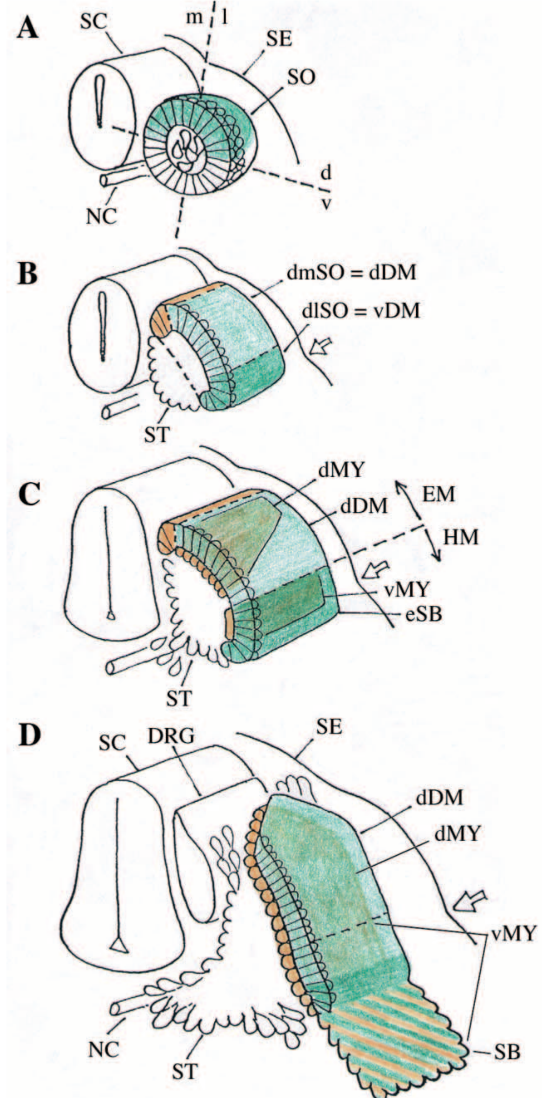


**Fig. 2.** Schematic presentation of thoracic somite development, mostly based on evidence from chicken and human embryos (Christ et al. 1983; 1986; Christ and Ordahl, 1995; Kaehn et al. 1988; Theiler 1957). *Pax3* expression (green) and *Myf5* expression (orange) are labelled. (A) Early epithelial somite. Dotted lines demarcate quadrants within the newly formed somite (SO). (B) Formation of sclerotome and dermomyotome after 'rotation' of the somite. Derivatives of the former dorsomedial and dorsolateral quadrants are now referred to as dorsal and ventral DM, respectively. (C) Formation of epithelial somitic bud and myotomes. Dorsal myotome (dMY) is generated from the anterior margin of the dorsal DM and will later generate dorsal (epaxial) muscle (EM). From the ventral DM margin, an epithelial somitic bud (eSB) forms which together with the ventral myotome independently generates the hypaxial musculature (HM). *Myf5* is first expressed in spatially separated dorsal and ventral myotomes. (D) De-epithelialization of the somitic bud (SB) and intermingling of its layers. The SB invades the ventrolateral body wall. Arrow indicates the border between back and ventrolateral body wall; d, dorsal; dm, dorsomedial; DM, dermomyotome; dl, dorsolateral; DRG, dorsal root ganglia; EM, epaxial muscle; HM, hypaxial muscle; l, lateral; m, medial; MY, myotome; NC, notochord; NT, neural tube; (e)SB, (epihelial) somitic bud; SC, spinal cord; SE, surface ectoderm; ST, sclerotome; SO, somite; v, ventral.

dermomyotome developed almost normally but that formation of dorsal myotome and dorsal dermomyotome was severely affected.

The described defects in *Pax3* and *Myf5* gene expression patterns in somites were observed in the same axial regions where previously we had noted abnormalities of the developing spinal cord. To evaluate spinal cord and somite defects in the same individual, mutant embryos were hybridized to both *Myf5* and *Wnt3a* probes. In wild-type embryos, *Wnt3a* was expressed in dorsal-most regions of the developing spinal cord including the roof plate (Fig. 1G,I,L). TS17 *opb* mutant embryos showed a narrower, patchy, and irregular shaped band of *Wnt3a* expression in the dorsal spinal cord within the lower cervical, thoracic and lumbar axial regions (Fig. 1H,K arrows). This dorsal spinal cord defect was even more pronounced at later stages such that several gaps in dorsal *Wnt3a* expression became evident (Fig. 1M, arrows). The same embryos analyzed in parallel for *Myf5* expression showed that the defects in dorsal myotome formation were detectable within the same axial regions as the abnormal *Wnt3a* expression in the neural tube (Fig. 1H). We, therefore, suggest that the two defects are causally linked (see Discussion).

### Loss of epaxial musculature in *opb* mutant embryos

The formation of dorsal (epaxial/deep back) musculature and ventral (hypaxial) musculature at later stages of development was observed by hybridization to myogenic marker genes. At TS20-21 (day 12.5 p.c.), the developing musculature strongly expressed *myogenin* and *Myod1;* whereas, *Myf5* was expressed weakly. In whole-mount in situ hybridizations and transverse sections of TS19 (day 11.5 p.c.) and TS20-21, the formation of dorsal muscles could be detected in wild-type embryos after hybridization with *Myf5* and *myogenin* probes. In *opb* mutant embryos, however, severe reduction and absence of dorsal muscles were observed; whereas formation of ventral musculature was not impaired (Fig. 3). In lower cervical and upper thoracic regions of *opb* mutant embryos, *myogenin* gene expression revealed a severe reduction in the formation of dorsal muscles (Fig. 3B) when compared to wild-type sections (Fig. 3A). Remnants of dorsal muscles were detectable in these axial regions (Fig. 3B) and are most likely derived from the flame-like dorsal myotome regions, observed in earlier stages (compare with Fig. 1D). In lower thoracic and lumbar regions almost no dorsal musculature was observed in *opb* mutants (Fig. 3D), whereas the formation of intercostal (Fig. 3B,D) and limb muscles (Fig. 3F) was not impaired. Similar observations were made after hybridization to *Myod1* (data not shown).

Whole-mount in situ hybridization of *Myf5* to TS19 *opb* embryos showed that ventral muscle anlagen had formed as in wild-type embryos (Fig. 3G,H); whereas, in dorsal regions of *opb* mutant embryos, only remnants and, in far dorsal positions, very faint stripes of *Myf5*-positive cells were detectable (Fig. 3H arrows) instead of the distinct dorsal muscle anlagen seen in wild type (Fig. 3G). *Myf5* expression in sections of TS20-21 *opb* embryos revealed that in lower cervical and upper thoracic regions, remnants of dorsal muscle could be observed (Fig. 3K); whereas, in lumbar and lower thoracic regions there was almost no dorsal muscle (Fig. 3M). In addition, in the loose dorsal mesenchyme, patches of *Myf5*-expressing cells were observed in the upper and lower thoracic axial regions of *opb* mutant embryos (Fig. 3K,M arrows). These dorsal patches of *Myf5*-positive cells were not observed after *myogenin* and *Myod1* hybridizations, and formation of muscle anlagen was never observed in these areas.

Together, these observations showed that the early defects in the formation of dorsal myotome in *opb* mutant embryos were later followed by the absence of dorsal (epaxial) muscles whereas ventral muscle anlagen were formed.

### Defects in dorsal sclerotome derivatives

To analyze possible defects in the developing sclerotome, we studied expression patterns of *Mox1* and *Pax1*. At TS17, *Mox1* expression in wild-type embryos can be detected in most somitic cells (except in differentiating myocytes; Fig. 4A) and becomes restricted at TS18 to presumptive connective tissue in muscle-skeleton appositions, the mesenchyme of the somitic buds, and to a layer of cells next to developing skeletal anlagen (Candia et al., 1992). After whole-mount in situ hybridizations, *Mox1* expression at TS19 outlined the developing neural arches dorsally and rib anlagen ventrally (Fig. 4C). In TS17 and TS19 *opb* mutant embryos, ventral expression domains of *Mox1* appeared normal (Fig. 4B,D) whereas dorsal domains of *Mox1* expression were severely reduced in lower cervical and upper thoracic regions (Fig. 4B arrowheads), or almost absent in lower thoracic and lumbar regions (Fig. 4B,D arrows).

Furthermore, changes in *Pax1* expression could be observed. During early somite differentiation, *Pax1* is expressed in the developing sclerotome and can later be detected in areas surrounding the condensing mesenchyme regions that start to shape the anlagen of the axial skeleton (Wallin et al., 1994). In TS17 wild-type embryos, strong *Pax1* expression in the sclerotome could be observed in a small anterior stripe and in the posterior half, extending more dorsally at its posterior margin (Fig. 4E). Weaker Pax1 signals were found in the ventral anterior half (Fig. 4E; Dietrich and Gruss, 1995; Wallin et al., 1994). In sections of TS20-21 wild-type embryos, very strong *Pax1* expression was observed in the mesenchyme around the anlagen of the vertebral bodies and in intervertebral disc anlagen (Fig. 4G; Wallin et al., 1994). In dorsal regions, strong *Pax1* hybridization signals could be detected in the mesenchyme surrounding the presumptive anlagen of the developing neural arches and neighboring perineural mesenchyme (Fig. 4G arrows). In *opb* mutant embryos, *Pax1* expression at TS17 showed that although the sclerotome formed in a segmented fashion, the pattern and level of expression were altered. In thoracic and lumbar regions, *Pax1* expression within each individual somite appeared much stronger, especially in anterior regions (Fig. 4F, arrows). In sections of TS20-21 *opb* embryos, *Pax1* expression was observed in ventral sclerotome regions but no hybridization signals in dorsal regions could be detected (Fig. 4H). In addition, *Pax1* expression in lateral sclerotome regions was elevated compared to wild type. Together, these observations demonstrate that in addition to defects in dorsal dermomyotome and myotome, abnormalities in the development of dorsal sclerotome derivatives could also be detected in *opb* mutant embryos.

The defects described for sclerotome derivatives can be expected to result, for example, in abnormalities of neural arches and perineural tissues. Indeed, in skeletal preparations

of the few embryos that survived until TS24 (day 15.5 p.c.), strong abnormalities in the developing laminae of the neural arches could be seen and minor malformations of the vertebral bodies and ribs were visible (Günther et al., 1994).

### Defects in *opb* mutant embryos during early stages of somite and spinal cord development

To examine earlier defects in *opb* mutants, we analyzed embryos at TS15 (day 9.5 p.c.). Aberrant gene expression patterns were clearly detectable at this stage in *opb* embryos after in situ hybridizations to *Pax3*-, *Myf5*- and *Wnt3a*-specific riboprobes in the developing neural tube and dorsal somites (Fig. 5). In the dorsal spinal cord of the thoracic body regions, *Pax3* expression occurred in an extremely narrow band in *opb* embryos (Fig. 5B,D) compared to wild type (Fig. 5A,C) and in addition, gaps in this band were obvious (Fig. 5D arrows). In the same axial regions, abnormalities of *Pax3* expression in the paraxial mesoderm were evident. In young somites, *Pax3* expression was first missing at the anterior and posterior margins. Shortly thereafter, *Pax3* expression retracted from dorsoanterior and dorsoposterior dermomyotome regions and was lost in the entire dorsal somite (Fig. 5B, arrows). In addition, the somites appeared to be truncated dorsally. Similarly, the expression pattern of *Wnt3a* in the dorsal spinal cord was much narrower and patchy in *opb* (Fig. 5F,H) than in wild-type embryos (Fig. 5E,G), and few gaps were apparent in the dorsal stripe of *Wnt3a* expression (Fig. 5F,H short arrows). In the same embryos, aberrant *Myf5* expression was visible in the developing dorsal myotomes along the same axial regions where the neural tube abnormalities occurred (Fig. 5F,H). In more advanced somites of wild-type embryos, the dorsal myotome begins to form at the dorsoanterior edge displaying a triangular shaped pattern of Myf5-positive cells (Fig. 5E arrowheads). In *opb* mutant embryos, however, only a central domain of strong *Myf5*-positive cells was observed in affected dorsal myotomes such that large gaps were evident between *Myf5*-positive cell clusters (Fig. 5F, arrowheads). Furthermore, in newly generated somites, *Myf5* expression in wild-type embryos could be first observed in a band of cells in the dorsomedial lip (Fig. 5E long arrow). In *opb* embryos, we could not detect this initial band of dorsomedial *Myf5*-positive cells (Fig. 5F long arrow).

In addition, expression of *Mox1* was studied as an early marker for formation and appearance of young somites. In wild-type embryos, expression of *Mox1* at TS15 could be observed throughout the early somite (Fig. 5I; Candia et al., 1992). Analysis of *Mox1* expression in *opb* mutant embryos revealed that somites in affected regions were tightly spaced along the anteroposterior axis (Fig. 5K). These results demonstrated that in *opb* embryos anterior and posterior somite regions do form and that the absence of *Pax3* and *Myf5* expression is not due to a general loss of cells but to a failure in induction or maintenance of *Pax3* and *Myf5* gene expression in these regions.

These observations suggest that in *opb* mutant embryos the defects in the differentiation of dorsal somitic mesoderm become established very early in development and will result in the loss of dorsal myotome and dermomyotome and subsequently in the absence of epaxial musculature and malformations of dorsal sclerotome derivatives.

## DISCUSSION

We show here that homozygous *opb* mutant embryos exhibit defects in derivatives of early dorsomedial somites as well as in dorsal spinal cord regions. In this respect, the *opb* mutation represents the only example so far of a mouse mutant in which both dorsal structures are affected. Although the primary defect is not known, our results support and extend previous observations that early interactions between the spinal cord and somites are required to properly specify the dorsomedial compartment in developing somites.

During normal development, the early dorsomedial region will generate dorsal myotome and dermomyotome, and the early dorsolateral region will give rise to ventral myotome and dermomyotome regions (Fig. 2). In *opb* mutant embryos, the formation of dorsomedial somite derivatives was strongly affected whereas dorsolateral derivatives developed almost normally.

Whole-mount in situ hybridizations of *opb* mutant embryos at TS15 and TS17 with *Pax3*- and *Myf5*-specific riboprobes showed that the dorsal myotome and dorsal dermomyotome did not differentiate, whereas ventral myotome and dermomyotome regions formed almost normally. In the same axial regions where dorsal somite deficiencies were observed, abnormal expression of *Wnt3a* and *Pax3* in the spinal cord was evident, indicating that significantly fewer cells in the neural tube acquired dorsal identities. Since affected somite and neural tube regions are in close proximity to each other (and to the surface ectoderm) our results suggest important interactions between the dorsal neural tube, dorsal somitic mesoderm and, possibly, surface ectoderm.

It has been shown that differentiation of dorsal somite derivatives depends on interactions of the early dorsomedial somite with neighboring tissues: neural tube, notochord and surface ectoderm (Ordahl and LeDouarin, 1992; Christ et al., 1992; Strudel, 1955). Therefore, it is very likely that specification of dorsomedial somite regions is influenced by a combination of signals from the adjacent neural tube and surface ectoderm (see Fan and Tessier-Lavigne, 1994) whereas dorsolateral regions become specified by a combination of signals from the adjacent lateral mesoderm (Hayashi and Ozawa, 1995; Pourquié et al., 1995) and surface ectoderm. These observations suggest that differentiation of dorsal muscle depends on specific signals from the neural tube. Our analysis of the *opb* phenotype further indicates that these signals are produced in dorsal or dorsolateral neural tube regions. The defects observed in opb mutant embryos could thus be explained in three different ways.

First, the primary defect in *opb* could reside in a defect to specify dorsal spinal cord regions. As a result, fewer cells in the neural tube acquire dorsal identities and signals that are normally emanating from the dorsal half of the neural tube, which are required to specify dorsomedial somite regions, are missing or are too weak in *opb* mutant embryos. As a consequence, dorsal myotome and dorsal dermomyotome are not differentiated properly. There is now evidence that the surface ectoderm is involved in specification of dorsal neural tube regions (Dickinson et al., 1995; Moury and Jacobson, 1990; Takada et al., 1994; Takahashi et al., 1992). Furthermore, the influence of surface ectoderm on induction and/or maintenance of the dermomyotome and marker gene expression in the dermomyotome has been described in several cases (Gallera,

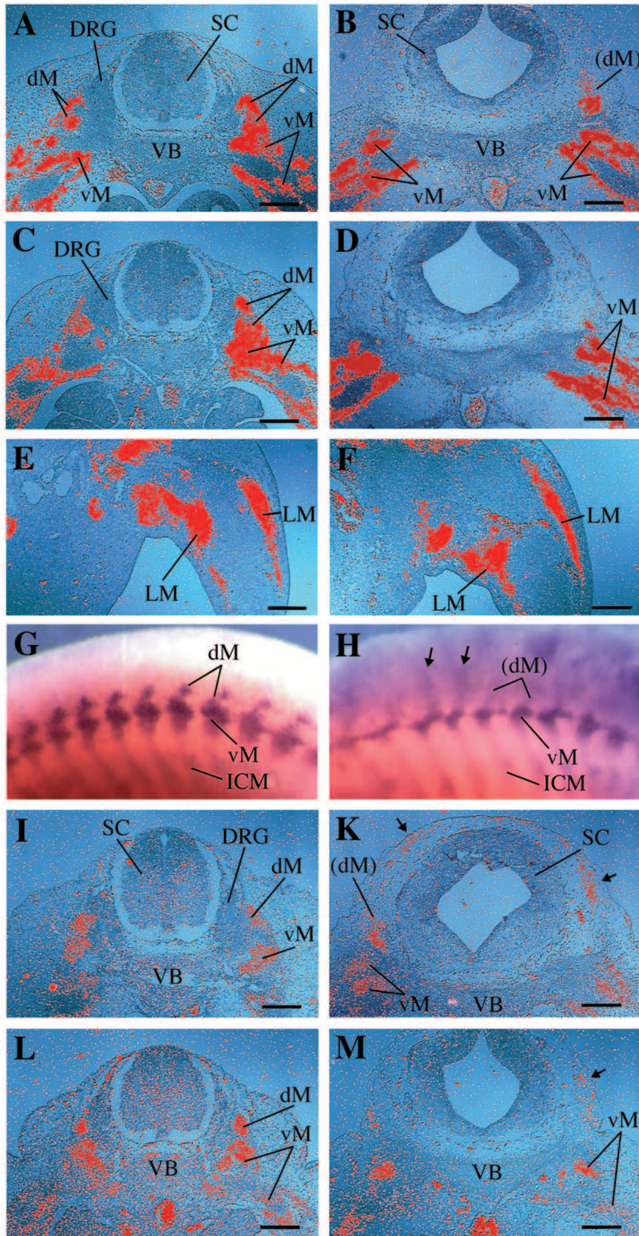


**Fig. 3.** Expression of *myogenin* and *Myf5* at TS19 and TS21 in wild-type and *opb* mutant embryos. In situ hybridizations of sections from (A,B) upper thoracic, (C,D) lower thoracic and (E,F) lumbar/hind limb axial regions of TS21 wild-type (A,C,E) and *opb* mutant (B,D,F) embryos, to *myogenin*. In *opb* mutant embryos, only remnants (B) or no dorsal muscle (D) were observed, whereas ventral body wall muscle (B,D) and limb muscles (F) formed normally. (G,H) lateral views of whole mount in situ hybridizations with *Myf5* riboprobes to TS19 wild-type and *opb* mutant embryos, respectively. Ventral musculature is forming in *opb* mutant embryos, but in dorsal regions faint stripes of *Myf5*-positive cells were seen (H, arrows) instead of the discrete anlagen of dorsal muscle in wild type (G). Sections from (I,K) upper thoracic and (L,M) lower thoracic regions of TS21 wild-type (I,L) and *opb* mutant (K,M) embryos after hybridization to *Myf5* riboprobes. In *opb* mutant embryos, discrete anlagen of dorsal muscle were not observed but patches of *Myf5*-positive cells were found in extreme dorsal regions (arrows). dM, dorsal muscle anlagen; DRG, dorsal root ganglion; ICM, intercostal muscle; LM, limb muscle; SC, spinal cord; VB, vertebral body; vM, ventral muscle anlagen. Scale bars, 160 µm.

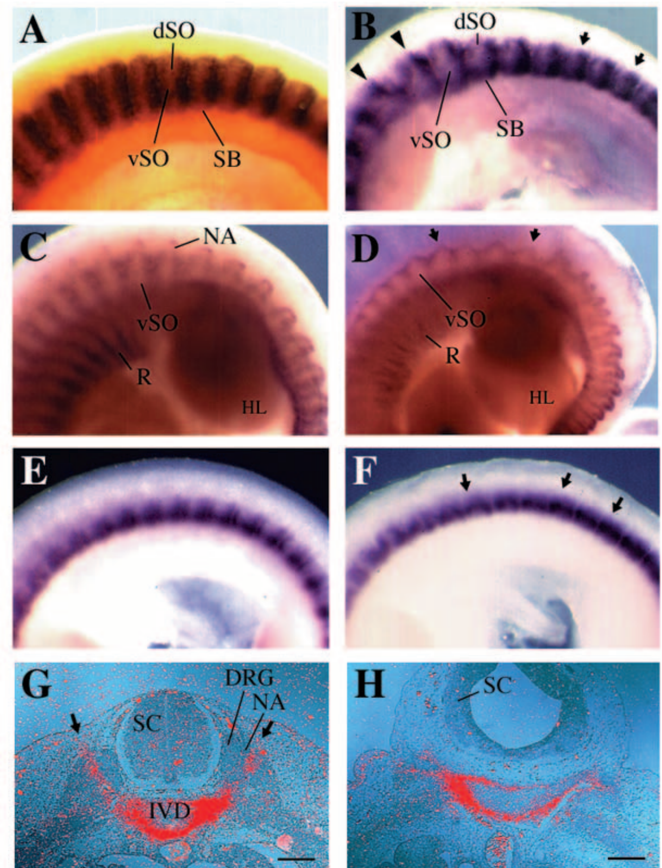


**Fig. 4.** Expression of *Mox1* and *Pax1* in sclerotome derivatives of wild-type and *opb* mutant embryos. Lateral views of whole mount in situ hybridizations of (A,C) wild-type and (B,D) *opb* mutant embryos at TS17 (A,B) and TS19 (C,D) after hybridization to *Mox1*. In *opb* mutant embryos, abnormal expression patterns of *Mox1* in dorsal regions were evident (arrows and arrowheads). (E,F) Lateral views of whole mount in situ hybridizations of TS17 wild-type (E) and *opb* mutant (F) embryos after hybridization to *Pax1*, respectively. In *opb* mutant embryos *Pax1* expression was stronger and appeared to extend more dorsally in the whole somite compared to wild type (arrows in F). (G,H) In situ hybridizations of sections from thoracic axial regions of TS21 wild-type and *opb* mutant embryos respectively to *Pax1*. Expression of *Pax1* in dorsal regions (arrows in wild type) was not observed in *opb* mutant embryos. dSO, dorsal somite; DRG, dorsal root ganglion; HL, hind limb; IVD, intervertebral disc; SB, somitic bud; SC, spinal cord; vSO, ventral somite derivatives. Scale bars, 160 µm.

1966; Fan and Tessier-Lavigne, 1994; Kuratani et al., 1994). Thus, a second possibility is that defects in surface ectoderm may be the primary cause for the *opb* phenotype in developing somites. Such ectoderm defects could then cause spinal cord and somite defects in parallel but independently. Third, it is possible that in *opb* embryos the dorsal somite defects are caused by a combination of both dorsal neural tube and surface ectoderm defects. At the moment, we cannot distinguish among these three possibilities and it will be necessary in the future to determine the tissue in which the primary defect occurs by in vitro culture assays. So far, we could not detect an apparent defect in the surface ectoderm when analyzing the expression pattern of the *Msx2* and *Wnt3a* genes in *opb* mutant

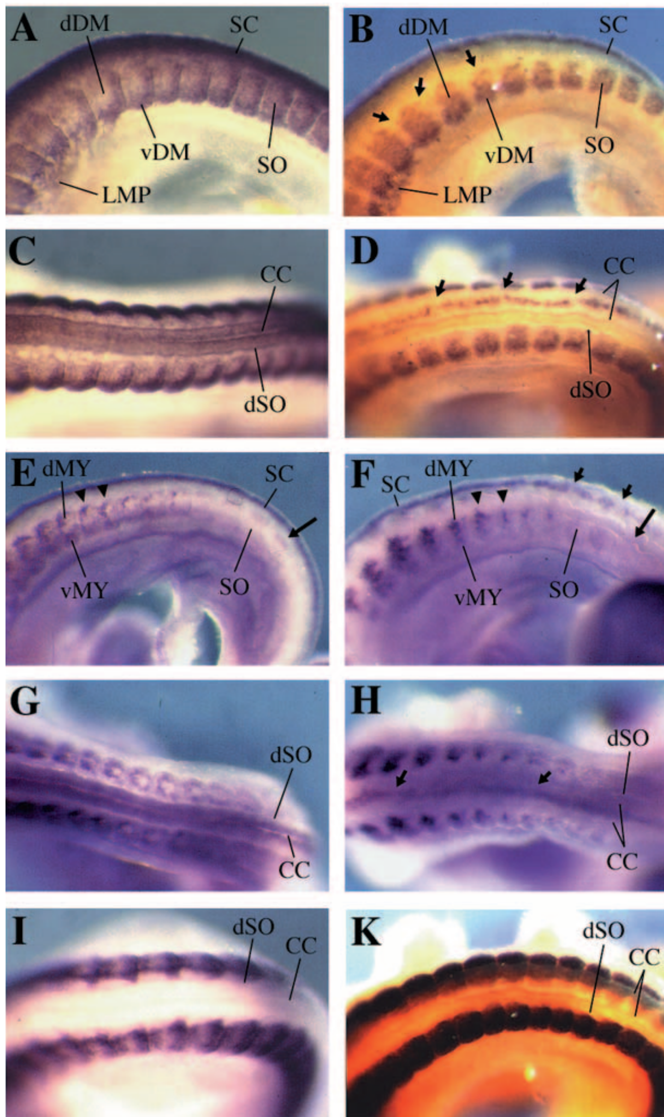


**Fig. 5.** Expression of *Pax3*, *Myf5*, *Wnt3a* and *Mox1* at TS15 after whole mount in situ hybridizations of wild-type and *opb* mutant embryos. (A,C) Wild-type and (B,D) *opb* mutant embryos after hybridization to *Pax3*; (A,B) lateral views, (C,D) dorsal views. (E,G) Wild-type and (F,H) *opb* mutant embryos after hybridization to both *Myf5* and *Wnt3a*; (E,F) lateral views, (G,H) dorsal views. (I,K) dorsolateral views of wild-type and *opb* mutant embryos respectively after hybridization to *Mox1*. *Pax3* gene expression in early somites of *opb* mutant embryos did not extend as far dorsally as in the wild type and was absent at the anterior and posterior margins (arrows in B). In the neural tube, *Pax3* expression was restricted to a much narrower band in *opb* compared to wild-type embryos, and in some regions, gaps of *Pax3* expression were visible (arrows in D). A similar observation was made for *Wnt3a* expression in the dorsal neural tube (arrows in F,H). *Myf5*-positive cells in the developing somite of *opb* mutant embryos (arrowheads in F) did not exhibit the typical triangular shape observed in wild-type embryos (arrowheads in E). In addition, the initial expression of *Myf5* in a band of cells in the dorsomedial somite region (long arrows in E,F) could not be detected in *opb* mutant embryos. CC, central canal (lumen of SC) d, dorsal; DM, dermomyotome; dSO, dorsal margin of somite; LMP, limb muscle precursor cells; MY, myotome; SC, spinal cord; SO, somite; v, ventral.

embryos (Günther et al., 1994). Although more marker genes need to be investigated, this observation makes it less likely that the primary defect resides within the surface ectoderm.

Recently, Buffinger and Stockdale (1995) have reported a low inducing activity of the dorsal neural tube to specify myogenic differentiation, and a strong inducing activity of the ventral neural tube. In their in vitro assay, however, they did not differentiate between induction of epaxial versus hypaxial musculature. We would thus suggest that the dorsal activity described in their transfilter assays may specifically induce dorsal muscle.

Almost nothing is known about possible influences of paraxial mesoderm and somites on the establishment of the dorsoventral axis in the neural tube. Williams and Ordahl (1994) observed that after extirpation of segmental plate mesoderm no changes in *Pax3* pattern in the neural tube could be observed. *opb* embryos, however, showed altered expression of *Pax3* expression in the spinal cord. Thus, it is less likely that in *opb* mutants the defect in patterning of the dorsoventral axis in the neural tube is caused by the abnormalities in somite differentiation.

The defects in myotome formation, which we observed in *opb* mutant embryos, demonstrate that epaxial and hypaxial musculature are not only derived from different embryonic cell lineages (Ordahl and Le Douarin, 1992) but also, that they can develop independently. In *opb* mutant embryos, derivatives of dorsal myotome regions (epaxial musculature) were absent, but all derivatives of ventral myotome regions and the somitic bud (hypaxial musculature) were generated. We propose that different signals or combinations thereof are involved in the specification of the two populations of skeletal musculature.

The dorsal sclerotome (presumptive neural arch anlagen and connective tissues including perineural tissues) originates from a region which, in early somites, is located at the border of dorsomedial and ventromedial somite compartments. It is, therefore, conceivable that the severe defects observed in the specification and/or differentiation of dorsomedial regions also affect the development of neighboring dorsal sclerotome regions. Alternatively, the dorsal sclerotome could also be directly specified by dorsal neural tube signals. These specification defects would result in the absence of dorsal *Mox1* and *Pax1* expression, and at later stages in strong malformations of dorsal sclerotome derivatives (Günther et al., 1994).

In conclusion, we describe a unique phenotype in *opb* mutant embryos in which the specification of dorsal spinal cord regions and derivatives of early dorsomedial somite regions is severely impaired. Our results suggest the presence of dorsal signals involved in the specification of dorsal neural tube regions and the formation of epaxial musculature. In the future, it will be important to describe the molecular basis of the *opb* mutation and to identify other gene(s) participating in the regulatory networks which control differentiation of the two different myogenic lineages in the developing mammalian embryo.

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