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Dmrt2 and *Pax3* Double-knockout Mice Show Severe Defects in Embryonic Myogenesis

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Myogenesis is one of the critical developmental processes in mammals. Several transcription factors from the dermomyotome affect embryonic myogenesis. Among these, Dmrt2 and Pax3 were tested for genetic and functional interactions during embryonic myogenesis by evaluating myogenin and desmin expression patterns in *Dmrt2–Pax3* mutant mouse embryos. In doubly homozygous mutant embryos, myogenin expression was reduced, and the expression pattern was altered dramatically. In *Pax3*-knockout mouse embryos, the pattern of Dmrt2 expression was altered, suggesting that Pax3 is important in maintaining the epaxial dermomyotome. Even though Pax3 and Dmrt2 are expressed in similar tissue- and developmental-stage-specific manners during dermomyotomal development, they appear to have independent roles in mammalian myogenesis. The processes characteristic of embryonic myogenesis are similar to those occurring during muscle regeneration in adults. Therefore, these results may provide insight into the pathogenesis of innate muscular dystrophy and may lead to the development of drugs to promote muscle repair after injury.

Abbreviations: BrdU, 5-bromo-2′ deoxyuridine; E, embryonic day; PBS, phosphate-buffered saline

In mammals, the vast majority of muscles are derived from somites, which are ball-shaped structures that are derived from the presomitic mesoderm during vertebral development and that differentiate into the dermomyotome and sclerotome. The dermomyotome develops into the dermatome and myotome. The dermatome differentiates into the back skin, the myotome differentiates into muscles, and the sclerotome ultimately differentiates into the bones of the body axis. Myotome formation in mammals is a complex process that involves an epithelial-to-mesenchymal transition, migration, and morphogenesis.[13] First, the cells located at the dorsomedial portion of the epithelial somites migrate between the dermomyotome and sclerotome to form the initial myotome. A secondary myogenesis wave occurs from the 4 boundaries of the dermomyotome during mammalian development. Once the migratory myoblasts reach their appropriate locations in the presumptive myotome, they elongate to span the entire rostrocaudal length of the somite. The limbs and trunk muscles are derived from the myotome of somites. This myogenic cell migration is dependent on scatter factor–hepatocyte growth factor, which is controlled by *Pax3* gene expression.[5,8,29] Mutation of *Pax3* causes defects in the muscles whose precursor cells migrate a long way, including muscles of the limbs.

Four muscle-specific transcription factors—Myf5, MRF4, MyoD, and myogenin—are essential for differentiation of muscle precursor cells.[15,21,24,32] These myogenesis-related transcription factors are expressed during the process of muscle development. Gene deletion experiments using knockout technology in mice have provided information on the function of the products of these genes. Deletion of the gene for a single myogenesis-related transcription factor does not produce muscle defects, but multiple gene deletions result in severe muscle defects during mammalian development.[1] In addition, each gene deletion causes skeletal defects of the vertebrae, suggesting linkage between muscle and bone development. Myocytes of the myotome excrete several secretion factors, including platelet-derived growth factors and fibroblast growth factors, and sclerotomal cells express their receptors. Pathways involving platelet-derived growth factors and fibroblast growth factors are believed to be responsible for the interaction between the myotome and sclerotome during mammalian development.[9,27]

Pax3 is one of the key component genes that are transcribed in muscle precursor cells, and Pax3 is known to regulate cell migration from the somite to the limb buds and intercostal areas. During somite development, Pax3 is expressed in the dermomyotome, which is the source of most myoblasts. A recent report describes *Pax3* gene expression in adult muscle stem cells, which are also known as satellite cells.[16] Mutation of *Pax3* causes muscle defects, particularly in limbs, in mammals.[28] Waardenburg syndrome type 3, which is due to homozygous mutation of *PAX3*, is characterized by a combination of hypoplasia or contracture of the limb muscles or joints, carpal bone fusion, and syndactyly in humans.[14] Overall, the patterning of myotomes is an important step during mammalian myogenesis. The expression of myogenesis-specific genes in the somites is severely decreased in the absence of proper myotomal patterning during somitogenesis. The community effect of myoblasts might be responsible for these phenotypes.[4] Each myoblast cell requires communication with others during myogenesis. Myoblasts can lose their myogenesis-specific gene expression programs if the cells are scattered.

The transcription factors Dmrt2 and Paraxis are expressed in the dermomyotome and function in myotome patterning. A null mutant of *Paraxis* showed defects in myotomal arrangement and vertebral development.[30] Null mutation of *Dmrt2* led to more severe defects in these same areas.[26] The myotome of the *Dmrt2* mutant had decreased expression of myogenesis-specific genes

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within the somites, and myotomal arrangement was severely distorted. The *Dmrt2* mutant had skeletal defects similar to those of mutants for the myogenesis-related transcription factor genes *Pax3* and *Paraxis*.

The interaction between Pax3 and Dmrt2 during myogenesis is interesting in view of the myogenesis-specific gene regulation and the migration control of myoblasts from the somites to the limb buds and intercostal muscles. The genes for these 2 factors are expressed in the same area, the dermomyotome, and at the same developmental stages. Moreover, Pax3 and Dmrt2 have a related function in muscle development, as shown by the similar phenotypes of each mutant. The present study examined the genetic and functional interactions between Pax3 and Dmrt2 by using double-null mutant mice.

Materials and Methods

Production of *Dmrt2*-null and *Pax3*-mutant mice. The generation of the *Dmrt2*-null mice has been described previously.[26] Briefly, AB1 embryonic stem cells were electroporated with the *Dmrt2*-targeting vector and selected in the presence of G418 and gancyclovir. Cell clones with correctly targeted homologous recombination at the *Dmrt2* locus were identified by Southern hybridization. Two positive clones were injected into mouse blastocysts, and chimeric mice were generated. After germline transmission, heterozygous mice were maintained by crossing them with C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME). The floxed neo cassette was deleted from the targeted locus by crossing heterozygous mice with BALB/c-TgN(CMV-Cre)1Cgn transgenic mice (Jackson Laboratory). Correct targeting at the *Dmrt2* locus was confirmed by Southern hybridization.[26] C57BL/6J-Pax3SP mutant mice were purchased from Jackson Laboratory and genotyped according to the vendor's protocol. $Pax3^{-/-}Dmrt2^{-/-}$ mice were produced through intercross mating of $Pax3^{+/-}Dmrt2^{+/-}$ mice.

The animals were maintained in conventional conditions. Mice received commercial food and water ad libitum. Artificial light was controlled under a 12:12-h light:dark cycle. The temperature of the room was 23 ± 1 °C, with 10 to 15 air changes hourly. This study was approved (no. 03-96-01233) and monitored by the Institutional Animal Care and Use Committee of the University of Texas MD Anderson Cancer Center (Houston, TX). The date when the vaginal plug was found was counted as embryonic day (E) 0.5. The pregnant mice were narcotized by CO_2 inhalation before necropsy. For each genotype, a minimum of 3 embryos per developmental time point were analyzed.

Whole-mount in situ hybridization. Nonradioactive whole-mount in situ hybridization was performed essentially as described elsewhere.[20] Briefly, the *myogenin* plasmid pMYOG-2, which contains the full coding region of the mouse *myogenin* gene, was linearized with *Hin*dIII and transcribed with T7 RNA polymerase (for antisense probes) or linearized with *Xba*I and transcribed with SP6 RNA polymerase (for sense control probes) according to the manufacturer's instructions for the preparation of nonradioactive digoxigenin transcripts (DIG RNA Labeling Mix, Roche, Mannheim, Germany). The plasmid pPAX3, which contains a partial cDNA of mouse *Pax3*, was linearized with *Hin*dIII and transcribed with T7 RNA polymerase (for antisense probes) or linearized with *Pst*I and transcribed with T3 RNA polymerase (for sense control probes). The plasmid pDMRT2-1 (containing the full coding region of mouse *Dmrt2*) was linearized with *Eco*RI and transcribed with T3 RNA polymerase (for antisense probes) or linearized with *Xba*I and transcribed with T7 RNA polymerase (for sense control probes). The embryos were fixed in phosphate-buffered saline (PBS) containing 4% paraformaldehyde and 0.2% glutaraldehyde, hybridized with each of the digoxigenin-labeled probes, and incubated with antidigoxigenin antibody (Antidigoxigenin-AP, Roche). Nitroblue tetrazolium and 5-bromo-4-chloro-3′-indolyphosphate p-toluidine were used for the color reactions. After whole-mount in situ hybridization, the selected embryos were prepared for cryosectioning by dehydration in 30% sucrose. The embryos were embedded in OCT resin (Sakura Fintek, Torrence, CA) or in a mixture of 7.5% gelatin in 15% sucrose[25] and frozen in liquid nitrogen. Sections (20 µm) were cut using a cryomicrotome (Leica, Heerbrugg, Switzerland). All the embryos evaluated were from E9.5 to E11.5, which is a critical period for embryonic myogenesis, as confirmed by *Dmrt2* mutational analysis.[26] All experiments were performed at least 3 times, and phenotypes were confirmed.

Histology. The immunohistochemistry of desmin was performed as follows: briefly, embryos fixed in 4% paraformaldehyde in PBS were dehydrated overnight in 30% sucrose in PBS at 4 °C and embedded in a solution containing 15% sucrose, 7.5% gelatin, and 0.05% azide; 20-µm cryosections then were prepared. After postfixation with cold acetone at 4 °C for 15 min, sections were washed with 0.3% Triton X100 in PBS; blocked for 1 h in PBS containing 5% bovine serum albumin, 0.3% Triton X100, and 0.05% azide; and incubated with antidesmin antibody (1:50 dilution in blocking solution; clone D33, Dako, Glostrup, Denmark) overnight in a humidified chamber at 4 °C. After being washed 3 times in PBS containing 0.3% Triton X100, the sections were blocked once more with the blocking solution just described. The slides were incubated with Alexa Fluor 488-labeled goat antimouse immunoglobulin G (1:800 dilution; Molecular Probes, Eugene, OR) at 4 °C for 4 h. After serial washing with PBS and counterstaining with 4′,6-diamidino-2-phenylindole, the slides were mounted and evaluated by fluorescent microscopy. The antibody for desmin was used because desmin is expressed throughout the cell body throughout myoblast development, making it a prominent marker for myocytes.

Terminal transferase-mediated dUTP nick end-labeling and 5-bromo-2′ deoxyuridine staining. Cell proliferation was analyzed by determining the amount of incorporation of 5-bromo-2′-deoxyuridine (BrdU; Sigma, St Louis, MO) into embryos, and the labeled cells in paraffin-embedded sections were detected using an immunohistochemical procedure with anti-BrdU antibody (Becton Dickinson, San Jose, CA). Briefly, pregnant mice were injected intraperitoneally with 100 µg BrdU/g body weight; concepti were recovered 2 h later. E10.5 and E11.5 embryos were processed for BrdU immunohistochemistry; they were fixed in 4% paraformaldehyde in PBS and infiltrated and embedded in paraffin. Serial sections (8 µm) were prepared and processed for staining according to the manufacturer's instructions (MOM Immunodetection Kit, Vector Laboratories, Burlingame, CA). Antigen retrieval was performed by boiling in 10 mM sodium citrate (Sigma) at pH 6.0 in a microwave oven for 20 min. The sections were blocked for 1 h in blocking reagent (Vector Laboratories) and incubated at room temperature in mouse anti-BrdU antibody (clone B44, Becton Dickinson) diluted 1:5 in diluent (Vector Laboratories). The sections were further incubated in MOM Biotinylated Antimouse immunoglobulin G (Vector Laboratories) according to the manufacturer's manual. Sections were stained with Fluorescein

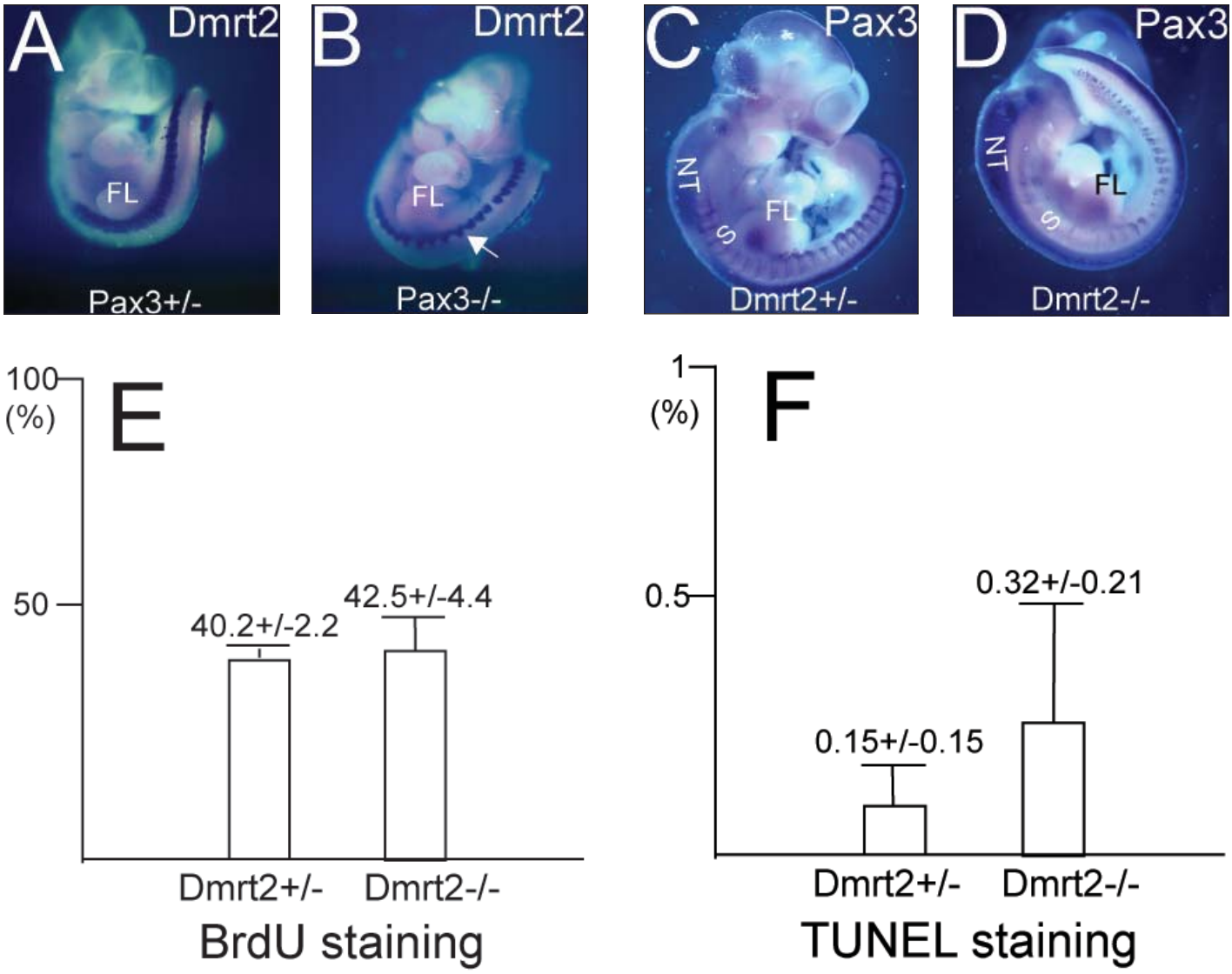

Figure 1. *Pax3* and *Dmrt2* expression in the *Dmrt2* null and *Pax3* homozygous mutant embryos and cell proliferation analysis of the dermomyotome in the *Dmrt2* null mutant. (A, B) *Dmrt2* expression in (A) $Pax3^{+/-}$ and (B) $Pax3^{-/-}$ embryos. The pattern of *Dmrt2* expression differs in the epaxial region (B, arrow). (C, D) *Pax3* expression in $Dmrt2^{+/-}$ and $Dmrt2^{-/-}$ embryos. (C) *Pax3* is expressed normally in the somites and neural tube. (D) *Pax3* expression in *Dmrt2* null mutant is changed markedly in somites of the interlimb region and mature somites around the forelimb region. *Pax3*-expressing cells are scattered and scarce in the mature somites. (E, F) The number of cells in dermomyotomal areas (dermatome, myotome) of the same size are represented as the percentage of positive cells per total number of cells. Six samples isolated from 3 different embryos were analyzed; data are given as mean ± standard error. All embryos are E10.5, except for those examined by terminal transferase-mediated dUTP nick end-labeling (E11.5). Because heterozygous *Dmrt2* and *Pax3* mutants were similar in phenotype to wild type, they were used as controls. FL, forelimb bud; NT, neural tube; S, somite.

Avidin DCS (Vector Laboratories) and counterstained with 4′,6-diamidino-2-phenylindole, and fluorescent cells were counted.

For the terminal transferase-mediated dUTP nick end-labeling assay, E10.5 and E11.5 embryos were fixed in 4% paraformaldehyde in PBS and processed for paraffin-embedded sections (8 µm). Antigen retrieval was performed by treating the slides with 20 µg/ml proteinase K (Sigma). After serial washing with PBS, the terminal transferase activity was examined using terminal transferase (Roche) with Chromatide Alexa Fluor 488-5-dUTP (Molecular Probes). After being counterstained with 4′,6-diamidino-2-phenylindole, the sections were evaluated under a fluorescent microscope. Total and positive cells within the same area were counted, and data were analyzed statistically.[17]

Results

***Pax3* homozygous mutants showed abnormal patterns of *Dmrt2* gene expression.** Embryos heterozygous for *Dmrt2* showed the same phenotype as that of wild-type embryos because the gene's effect is recessive.[26] The *Pax3* mutation was also recessive. Therefore, heterozygous embryos were selected as the controls and compared with embryos with null mutations. *Pax3* transcripts were present in the dermomyotome (Figure 1 C), as previously reported;[26] and *Dmrt2* expression occurred in the same area at the same developmental stages (Figure 1 A). Both genes showed similar expression patterns in somites, and both null mutants exhibited similar phenotypes, such as vertebral and rib malformations.

These results suggest that the products of both genes have similar or genetically related functions during somitogenesis. *Dmrt2* expression in *Pax3* mutant mice had not been reported previously.

Dmrt2 expression in *Pax3* mutants was slightly different than that of *Pax3* expression in *Dmrt2* null mutants (Figure 1 B, D). *Dmrt2* expression was present in the mature somites of the *Pax3* mutant and was altered dramatically in the dorsal (epaxial) region of the *Pax3* homozygote (Figure 1 B, arrow). The *Dmrt2* gene expression pattern of the ventral (hypaxial) region within the somites of the *Pax3* homozygous mutant was similar to those of wild-type and heterozygous embryos. In addition, *Pax3* expression in the *Dmrt2* null mutant was altered mainly in mature hypaxial somites (Figure 1 D). Almost no *Pax3* expression was present in the mature somites of the forelimb bud region. This expression pattern may be due to scattering of *myogenin*-expressing cells because the cell survival and proliferation of mature somites did not differ significantly between *Dmrt2* null mutants and heterozygotes (Figure 1 E, F).

Myogenin expression was normal in *Pax3*$^{+/-}$*Dmrt2*$^{+/-}$ embryos. Because myogenin is expressed by myoblasts and is essential to myogenesis, *myogenin* gene expression has been used as a marker for myogenic cells during mammalian development. Expression of the *myogenin* gene in the myotome was decreased dramatically in both *Dmrt2* null and *Pax3* homozygous mutant embryos. *Pax3*$^{+/-}$*Dmrt2*$^{+/-}$ mutants might show myogenic defects if these genes are genetically linked or closely associated. However, *myogenin* expression in *Pax3*$^{+/-}$*Dmrt2*$^{+/-}$ embryos was comparable with that in wild-type embryos (Figures 2 B, 3 A, and 4 A, B). In particular, *myogenin* expression in the somites of the interlimb and limb bud areas seemed normal.

The arrangement of myogenic cells in the interlimb somites of *Dmrt2* and *Pax3* homozygous mutants was not changed severely by further decreases in the level of *Pax3* or *Dmrt2* expression. The arrangement of the myogenic cells of the interlimb somites in the *Dmrt2* homozygous mutants was abnormal (Figure 2 C). Cells expressing *myogenin* were present in the intersomite area, and the typical arrangement of myogenic cells was disrupted in the *Dmrt2* mutant (Figure 2 C, arrowhead). The mutant with decreased *Pax3* expression showed a similar phenotype (Figure 2 D, arrowhead). These results indicate that the level of *Pax3* expression does not affect the function of Dmrt2 in somite development in the interlimb region. The somites of the interlimb region of the *Pax3* homozygous mutant also contained altered myogenic cells (Figure 2 E, arrowhead). In particular, *myogenin*-expressing cells were not confined within the somite boundary in the hypaxial myotome (Figure 2 A, E). This phenotype was not changed as a result of further decreases of *Dmrt2* expression (Figure 2 E, F).

The forelimb region of the *Pax3*$^{+/-}$ *Dmrt2*$^{-/-}$ mutant showed decreased expression of *myogenin*. In the hindlimb regions, the expression pattern of *myogenin* in *Pax3*$^{+/-}$ *Dmrt2*$^{-/-}$ embryos was equivalent to that of *Pax3*$^{+/-}$ *Dmrt2*$^{+/-}$ embryos (Figure 3 A, C, arrowhead). However, *myogenin* expression in the forelimb region of *Pax3*$^{+/-}$ *Dmrt2*$^{-/-}$ embryos at E11.5 was markedly reduced (Figure 3 A, C, arrow). *Pax3* homozygotes showed abnormal myogenic cell migration in both limb buds, as previously reported (Figure 3 B, arrow and arrowhead), and *Dmrt2* null embryos (*Pax3*$^{+/+}$ *Dmrt2*$^{-/-}$) had no defects in this region (Figure 2 C, arrow).

A possible hypothesis is that the depletion of Dmrt2 destroys the normal structure of the mature dermomyotome, which is the forelimb region, and consequently alters how *Pax3* regulates cell migration in E11.5 embryos. Indeed, *Pax3* expression in the forelimb region was severely decreased in mature somites of E10.5 to E11.5 *Dmrt2* mutants (Figure 1 D). These possibilities were examined by staining of the forelimb region for desmin, a marker for myogenic cells. In *Pax3*$^{+/-}$ *Dmrt2*$^{-/-}$ embryos, desmin-expressing cells were scattered widely laterally and ventrally and showed relatively weak desmin expression (Figure 3 D through G). Therefore, Dmrt2 likely influenced the typical pattern of formation of mature somites at this stage, subsequently temporarily decreasing *myogenin* expression in the forelimb region.

***Pax3*$^{-/-}$*Dmrt2*$^{-/-}$ mutants showed severe myogenic defects.** Some types of *Pax3* homozygous mutations are lethal to mouse embryos,[7] but most, including that used in this experiment, live until E11.5. In addition, *Dmrt2* null mutants were alive at E18.5, but *Pax3*$^{-/-}$*Dmrt2*$^{-/-}$ embryos died earlier than day E10.5. The cell-migration defects of the myocytes could not be tested because myogenic cell migration occurs later than E10.5 in mouse embryos. Three independent experiments showed that every *Pax3*$^{-/-}$*Dmrt2*$^{-/-}$ embryo exhibited suspended development at approximately the E9.5 and E10.5 stages. This finding suggests that the loss of function of both gene products causes greater malformation or earlier embryonic death than does the absence of either single factor.

The level of *myogenin* gene expression was markedly decreased in *Pax3*$^{-/-}$*Dmrt2*$^{-/-}$ embryos (Figure 4), and the pattern of the *myogenin*-expressing cells was distorted dramatically, more so than in either the *Dmrt2* null or *Pax3* homozygous mutants. However, *myogenin* gene expression in the *Pax3*$^{-/-}$*Dmrt2*$^{-/-}$ embryos was not completely abolished, although the number of *myogenin*-expressing cells was considerably lower than that of the *Pax3*$^{+/-}$ *Dmrt2*$^{+/-}$ embryos or other compound mutants at similar stages (Figure 4 C through E). Because discriminating epaxial from hypaxial myotomes is difficult at this young stage, the area affected *Pax3*$^{-/-}$ *Dmrt2*$^{-/-}$ embryos could not be established definitively.

Discussion

The epaxial and hypaxial regions of the dermomyotome are under different genetic control mechanisms. In particular, the Pax3 function in the dermomyotome itself has not been investigated well. The role of Pax3 in the epaxial dermomyotome was revealed through the abnormal expression pattern of *Dmrt2* in the *Pax3* homozygote (Figure 1 B). Pax3 may be important for maintaining the epaxial dermomyotome. *Pax3* is a marker gene for the myogenic precursor cells that reside in the dermomyotome of embryos and in adult muscles. *Dmrt2* also regulates myogenesis from the dermomyotome, which is the source of most myotomal cells during mammalian development. Given the expression patterns of these genes in mutants, each gene (*Pax3* and *Dmrt2*) seems to be regulated genetically independently during somitogenesis, because *Dmrt2* is still expressed in the dermomyotome of the *Pax3* mutant and vice versa. A double mutant of these 2 genes can provide valuable information about the myogenesis process in mammalian development.

Myogenin expression was reduced markedly in *Pax3*$^{-/-}$*Dmrt2*$^{-/-}$ embryos, which also showed altered arrangement of *myogenin*-expressing cells. Except for the very first myoblast formed (the 'pioneer myoblast'), every myogenic cell in the body trunk is derived from the dermomyotome.[13] Without expression of *Pax3* and *Dmrt2*, myogenic cells can arise from the dermomyotome but cannot arrange themselves appropriately within the somites. One of

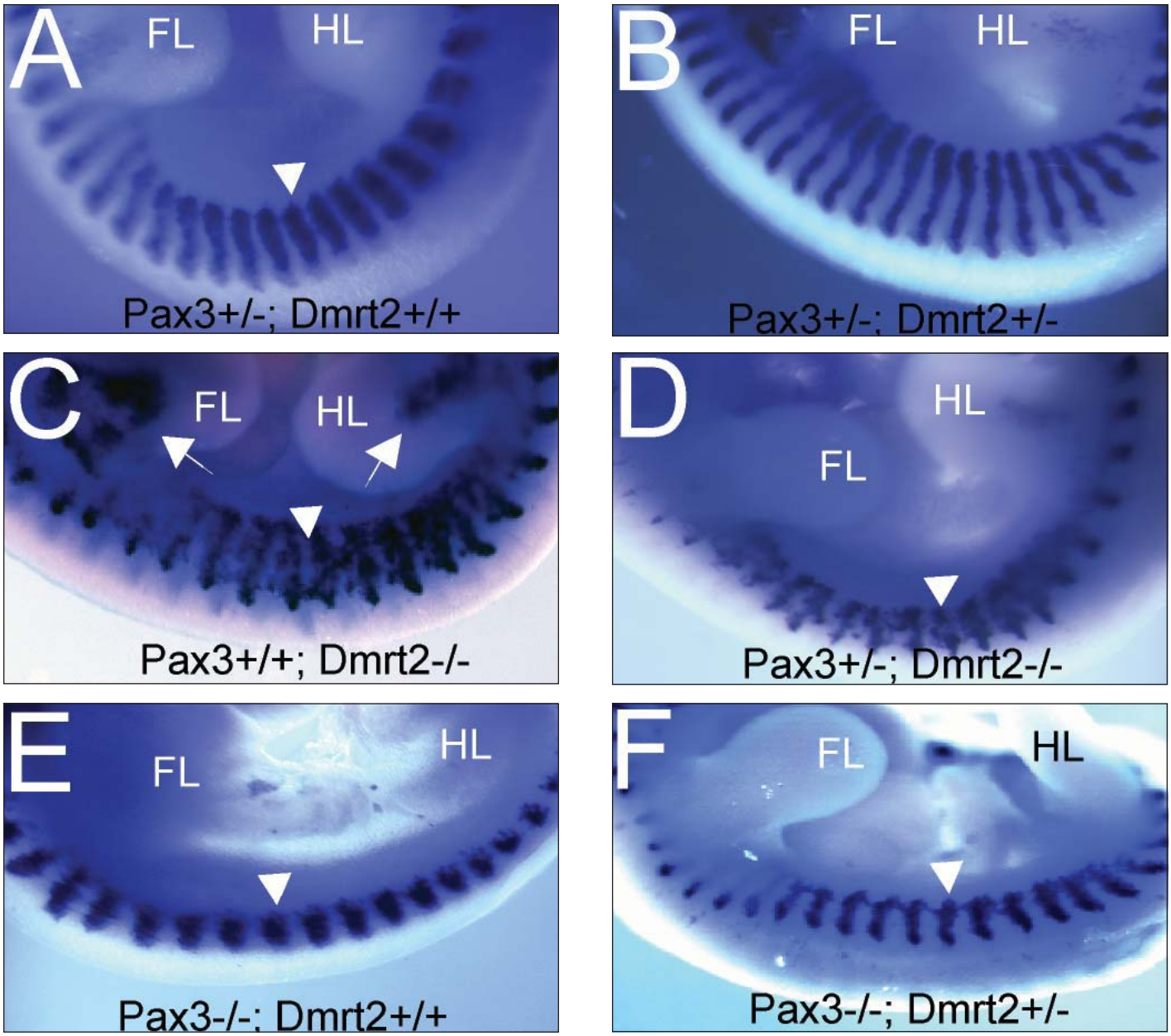

Figure 2. *Myogenin* expression in various types of *Pax3* and *Dmrt2* mutants. (A, B) *Myogenin* expression was present within the somites and limb buds of (A) $Pax3^{+/-}$ $Dmrt2^{+/+}$ and (B) $Pax3^{+/-}Dmrt2^{+/+}$ embryos. (C, D) *Myogenin* expression in (C) $Pax3^{+/+}$ $Dmrt2^{-/-}$ and (D) $Pax3^{+/-}$ $Dmrt2^{-/-}$ embryos. (E, F) *Myogenin* expression in (E) $Pax3^{-/-}$ $Dmrt2^{+/+}$ and (F) $Pax3^{-/-}$ $Dmrt2^{+/-}$ genotypes. The myogenic cell arrangement within the somites of the *Dmrt2* mutant is changed but not abolished due to decreased expression of *Pax3* (C, D, arrowhead). *Myogenin*-expressing cells are scattered in the hypaxial region of the *Pax3* mutant, but the arrangement of the somites is not severely altered by the decreased expression of *Dmrt2* (E, F, arrowhead). Both limb buds of the *Dmrt2* null mutant contained *myogenin*-expressing cells (C, arrow). All the embryos analyzed were approximately E11.5. FL, forelimb bud; HL; hind-limb bud.

functions of *Dmrt2* is to maintain somite patterning by regulating the components of the extracellular matrix, such as laminin 1.[26] Laminin 1 is expressed throughout the dermomyotome, including the dermatome and myotome.[11,31] Expression of the laminin 1 protein is confined within the dermomyotome and is dissociated in the migration sites of the myotomes. The dissociation of laminin in the myotome is also essential to myocyte migration, which occurs in a portion of the area of strong *Pax3* expression in the dermomyotome.[2]

Myogenic cell migration from the somites to the limbs is mediated in part by the scatter factor–hepatocyte growth factor pathway, which is regulated by *Pax3* expression. In $Pax3^{+/-}$ $Dmrt2^{-/-}$ embryos, very weak signals of *myogenin* were present near the forelimb region; desmin expression was weak also. The *Dmrt2* null mutation causes dissociation of the mature dermomyotome (forelimb region) during mouse development at approximately E10.5 to E11.5.[26] This effect may explain why the forelimb was affected more severely than the hindlimb at this developmental

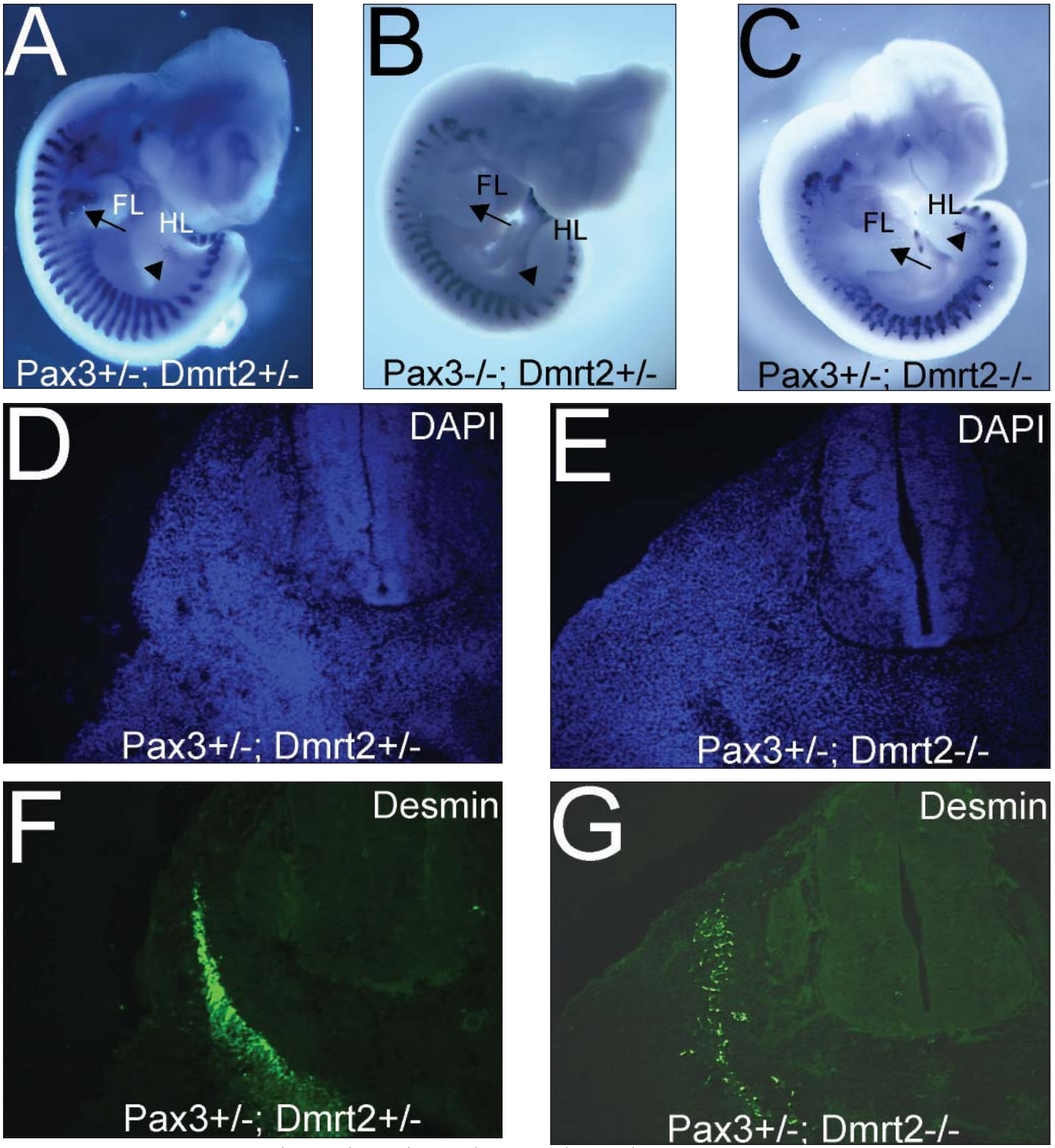

Figure 3. *Myogenin* expression in *Pax3*$^{+/-}$ *Dmrt2*$^{+/-}$, *Pax3*$^{-/-}$ *Dmrt2*$^{+/-}$, and *Pax3*$^{+/-}$ *Dmrt2*$^{-/-}$ embryos, and desmin expression in the forelimb region of the *Pax3*$^{+/-}$ *Dmrt2*$^{-/-}$ mutant. *Myogenin* expression is present in the limbs of the *Pax3*$^{+/-}$; *Dmrt2*$^{+/-}$ genotype (A, arrow and arrowhead). (B) The *myogenin* expression of both limb buds is severely decreased in the *Pax3* null mutant (arrow and arrowhead). (C) *Myogenin* expression of the forelimb bud is unclear in *Pax3*$^{+/-}$ *Dmrt2*$^{-/-}$ embryos (arrow), but that in the hindlimb bud is clear (arrowhead). (D, F) Desmin expression in the forelimb bud region and 4′,6-diamidino-2-phenylindole staining in *Pax3*$^{+/-}$ *Dmrt2*$^{+/-}$ embryo. (F) The desmin-expressing cells are growing near the medioventral portion of the embryos. (E, G) Desmin expression in the forelimb region and 4′,6-diamidino-2-phenylindole staining of the *Pax3*$^{+/-}$; *Dmrt2*$^{-/-}$ embryo. Desmin-expressing cells are present but scattered and have very weak signals (G), which coincide with those in (C). All the embryos were around E11.5; the width of each photo in panels D through G is equivalent to 16.6 μm; FL, forelimb bud; HL, hindlimb bud.

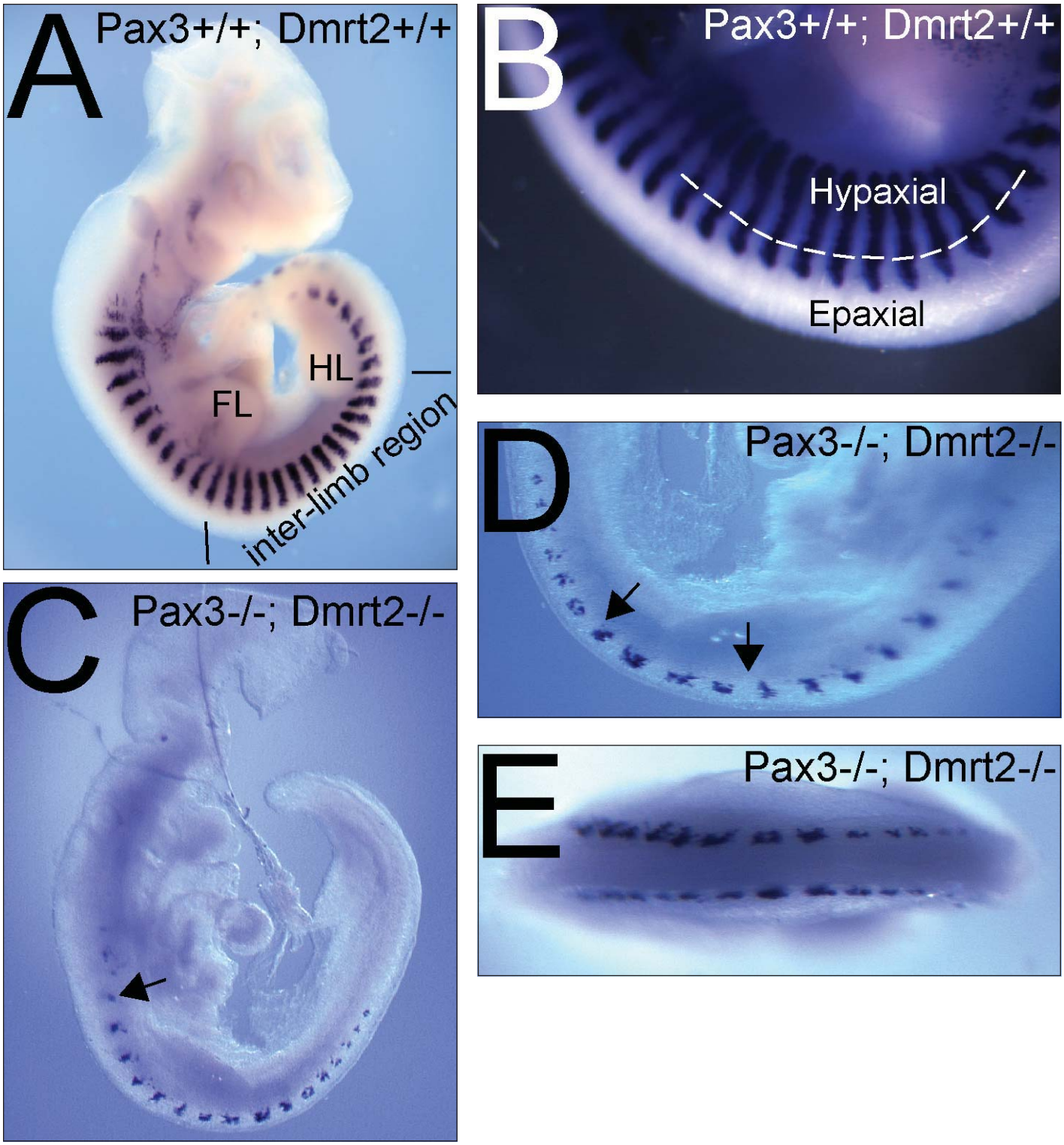

Figure 4. *Myogenin* expression in the *Pax3*$^{-/-}$ *Dmrt2*$^{-/-}$ mutant compared with the wild type. (A, B) Wild-type embryos show clear *myogenin* expression within the somites and limb buds, similar to that in *Pax3*$^{+/-}$ *Dmrt2*$^{+/-}$ embryos. (C–E) Embryonic development is arrested between E9.5 and E10.5. (D) Close-up of the interlimb region. (E) Dorsal view of (D). FL, forelimb bud; HL, hindlimb bud.

stage and why this phenotype was temporal. Dissociation of the basal lamina of the dermomyotome is needed for the migration of myogenic cells from the somites to the limb buds.[2] However, near absence of laminin expression can accelerate migration and thus scatter myogenic cells within the somites and limb buds. Because of the community effect, scattered myogenic cells cannot maintain specific gene expression.[4]

Many alternative pathways during embryonic muscle devel-

opment are unknown as yet. Many muscle-specific transcription factors, including *Pax3*, *Myf5*, *MRF4*, *MyoD* and *myogenin*, are involved in the development of embryonic muscle cells. In addition, Six1 and Six4 regulate myogenesis through Pax3 regulation;[10] their gene regulations are closely related. In the absence of 1 or more of these muscle-specific transcription factors, myogenic cells can adopt alternative pathways during embryogenesis. Some double mutations of these transcription factors can abolish a part of muscle development but not all of it. However, *Dmrt2* and *Paraxis* have been suggested to regulate the formation of the myotomal pattern within the somites, and disruptions of this patterning can result in malformation of the embryonic myogenesis program.[30] The current study revealed severe myogenic defects in mouse embryos mutant for both *Pax3* and *Dmrt2*. However, double-null mutation of these 2 genes did not abolish embryonic myogenesis completely. Signals from the dorsal neural tube, dorsal ectoderm, and lateral plate mesoderm can stimulate the development of the dermomyotome and myotome, which is mediated in part by the Wnt and BMP proteins.[4,6,12,18,19,23] Therefore, this alternative stimulation may enable the myogenic precursor cells within the somites to survive in the environment of the dermomyotome under unfavorable conditions such as double mutation of *Dmrt2* and *Pax3*.

Cells that express Pax7 and Pax3 are known as adult skeletal muscle precursor cells even though they do not express myogenesis-specific genes such as MRFs.[22] In mammalian development, all muscle precursor cells (except for those for various parts of the head muscles) are derived from somites. In adult muscles, satellite cells are considered to be the stem cells for muscle regeneration after a muscle injury.[3] in mammalian somitogenesis, *Pax3* expression is similar to that of *Dmrt2*, which also is expressed in adult muscles, on the basis of Northern analysis (data not shown). Therefore, *Pax3* and *Dmrt2* may play a role in the muscle regeneration process of adult muscles, but more study is needed.

Acknowledgment

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