LETTERS

A Pax3/Pax7-dependent population of skeletal muscle progenitor cells

Frédéric Relaix[1], Didier Rocancourt[1], Ahmed Mansouri[2] & Margaret Buckingham[1]

During vertebrate development, successive phases of embryonic and fetal myogenesis lead to the formation and growth of skeletal muscles[1]. Although the origin and molecular regulation of the earliest embryonic muscle cells is well understood[2], less is known about later stages of myogenesis. We have identified a new cell population that expresses the transcription factors Pax3 and Pax7 (paired box proteins 3 and 7) but no skeletal-muscle-specific markers. These cells are maintained as a proliferating population in embryonic and fetal muscles of the trunk and limbs throughout development. Using a stable green fluorescent protein (GFP) reporter targeted to *Pax3*, we demonstrate that they constitute resident muscle progenitor cells that subsequently become myogenic and form skeletal muscle. Late in fetal development, these cells adopt a satellite cell position characteristic of progenitor cells in postnatal muscle. In the absence of both Pax3 and Pax7, further muscle development is arrested and only the early embryonic muscle of the myotome forms. Cells failing to express Pax3 or Pax7 die or assume a non-myogenic fate. We conclude that this resident Pax3/Pax7-dependent progenitor cell population constitutes a source of myogenic cells of prime importance for skeletal muscle formation, a finding also of potential value in the context of cell therapy for muscle disease.

During the onset of skeletal myogenesis in the embryo, Pax3 is required for the survival of the ventro-lateral dermomyotome, the part of the somite that gives rise to hypaxial body[3,4] and limb[5] musculature. Pax3 is also implicated in the determination of myogenic cell fate, acting through MyoD[6]. In the absence of the myogenic regulatory factors MyoD, Myf5 and Mrf4 (ref. 7), skeletal muscle does not form and no myogenic cells are present[8]. Most of the functions of *Pax3* can be replaced by its paralogue *Pax7* (ref. 9). However, *Pax7*, which is also expressed in the somite, is only essential for myogenesis after birth, when it is expressed in satellite cells (the progenitors of adult skeletal muscle)[10,11].

Pax3 is first expressed in the presomitic mesoderm and this expression is maintained in the somitic epithelium of the dermomyotome[12–15] (Fig. 1a). However, at embryonic day (E)10.5, Pax3 is also detected (by immunohistochemistry) in the myotome, the first skeletal muscle to form[16] (Fig. 1b, c). In less mature posterior somites, this expression is first observed in a few cells lying under the epithelial dermomyotome (Fig. 1a), whereas in more mature anterior somites, Pax3-expressing cells are found throughout the myotome (Fig. 1b, c). The number of Pax3-positive ($Pax3^+$) myotomal cells increases as the central dermomyotome loses its epithelial structure (Fig. 1d (middle and right panels) and e), suggesting that they arise directly from the central dermomyotome. Similar results were observed for Pax7, which is first expressed in the central dermomyotome[9,17] (Supplementary Fig. S1a) and then co-localizes with Pax3-positive cells in the myotome (Fig. 1e, right panel), where about 87% of cells are $Pax3^+Pax7^+$, 10% are $Pax3^+$ only, and 3% are $Pax7^+$ only. Recent experiments in the chick embryo show that $Pax3^+Pax7^+$ cells in the myotome derive from the dermomyotome[25]

At E10.5, expression of the myogenic determination factor Myf5, visualized as β-galactosidase (β-gal) from a $Myf5^{nlacZ}$ allele, is seen in the lips of the dermomyotome[16], which also express high levels of Pax3 (arrows in Fig. 1d). In contrast, most $Pax3^+Pax7^+$ cells in the myotome do not co-express *Myf5* (about 93% are $Myf5^-$) (Fig. 1d and Supplementary Fig S1a). This lack of co-expresison is also seen for other markers of cell engagement in the myogenic programme, such as desmin (Supplementary Fig. S1b) or MyoD (Fig. 1f). No co-expression was detected for sarcomeric myosin heavy chain (MyHC) (Fig. 1g), which marks differentiated cells.

The $Pax3^+Pax7^+$ population shows labelling with a mitotic marker (Fig. 1h), indicating the presence of dividing cells. We examined the proliferation of this population compared to the myogenic cells in the myotome, using co-localization with cyclin A, a marker of S and G2 phases (which represent about 50% of the cell cycle length). Data presented in Fig. 1m–o, bottom panels and quantified in Fig. 1p show that about 96% of the $Pax3^+$ cells in the dermomyotome are dividing, as are 81% of the $Pax3^+Pax7^+$ progenitors in the myotome. In contrast, less than 20% of the $MyoD^+$ cells in the myotome are proliferating (Fig. 1o, bottom panel) and only 8% of the $Myf5(\beta\text{-gal})^+$ cells are proliferating (Fig. 1n, bottom panel), although this lower figure for Myf5 probably reflects β-gal stability from the $Myf5^{nLacZ}$ allele with labelling of some differentiating muscle cells. Of the total number of cyclinA-positive cells in the myotome, 76% are $Pax3^+$ (Fig. 1m, lower panel). We conclude that the $Pax3^+Pax7^+$ cells constitute the main proliferating population of the myotome. Cells that are not detected as proliferating might reflect heterogeneity in the cell population, or might correspond to cells that are progressing into the myogenic programme, but for which Pax3 expression is still detectable.

Analysis of skeletal muscle masses at later stages shows that the $Pax3^+Pax7^+$ population persists and continues to be distinct from cells that have entered the myogenic programme. MyoD, which marks the myogenic cells, does not co-localize with Pax7, as determined either by staining with an antibody recognizing Pax7 (Fig. 1i, right panel) or by a more sensitive assay using an antibody to β-gal generated from the $Pax7^{LacZ}$ allele[18] (Fig. 1j, right panel). Similar results were obtained at all stages examined (E11.5–E17.5; Figs 2, 3, Supplementary Fig. S1 and data not shown). At later stages, $Pax3^+Pax7^+$ cells are also actively dividing (Fig. 1k, right panel). They are also clearly distinct from connective tissue, which is labelled using an antibody against T-cell factor 4 (TCF4) (ref. 19; Fig. 1l, right panel).

Having identified a novel $Pax3^+Pax7^+$ population of proliferating cells distinct from the myogenic cells of skeletal muscle, we then investigated whether this population contributes to the growth of

[1]C.N.R.S. URA 2578, Department of Developmental Biology, Pasteur Institute, 75724 Paris Cedex 15, France. [2]Max-Planck Institute for Biophysical Chemistry, Department of Molecular Cell Biology, D-37077 Gottingen, Germany.

this tissue. We took advantage of the fact that the products of different reporter genes targeted to the *Pax3* and *Pax7* loci[18] have different stabilities (Fig. 2s and Supplementary Fig. S2). GFP, encoded by the reporter sequence that we targeted to *Pax3* (F.R and M.B., unpublished data), is more stable than β-gal generated from the $Pax3^{IRESnLacZ/+}$ or $Pax7^{LacZ/+}$ alleles[18,20], which has comparable stability to the endogenous proteins (Supplementary Fig. S2). Most (93%) of β-gal$^+$ cells detected in trunk muscles of $Pax7^{LacZ/+}$ mice at E13.5 or E15.5 do not co-express desmin (Fig. 2a–d, j–l), but they do co-localize with the GFP$^+$ cells generated from the $Pax3^{GFP/+}$ allele (Fig. 2e). However, there are additional GFP$^+$ cells that are not β-gal$^+$ (Fig. 2e), and all of these co-localize with desmin at E13.5 (Fig. 2f). Similar results were obtained at E15.5, when multinucleated muscle fibres are clearly both GFP- and desmin-positive (Fig. 2m–o), in contrast to some individual cells labelled only with GFP (arrows in Fig. 2m–o). In addition, nearly all (87%) of the Pax7$^+$ cells are dividing, as shown by co-expression of Ki67, which marks cycling cells (Fig. 2g–i), whereas only 9% of the GFP$^+$/Pax7$^-$ cells co-express Ki67 (data not shown). At this stage, 89% of cycling cells located within the muscle masses express Pax7, demonstrating that the Pax3$^+$/Pax7$^+$ progenitors constitute the main proliferating cell population (Fig. 2g–i).

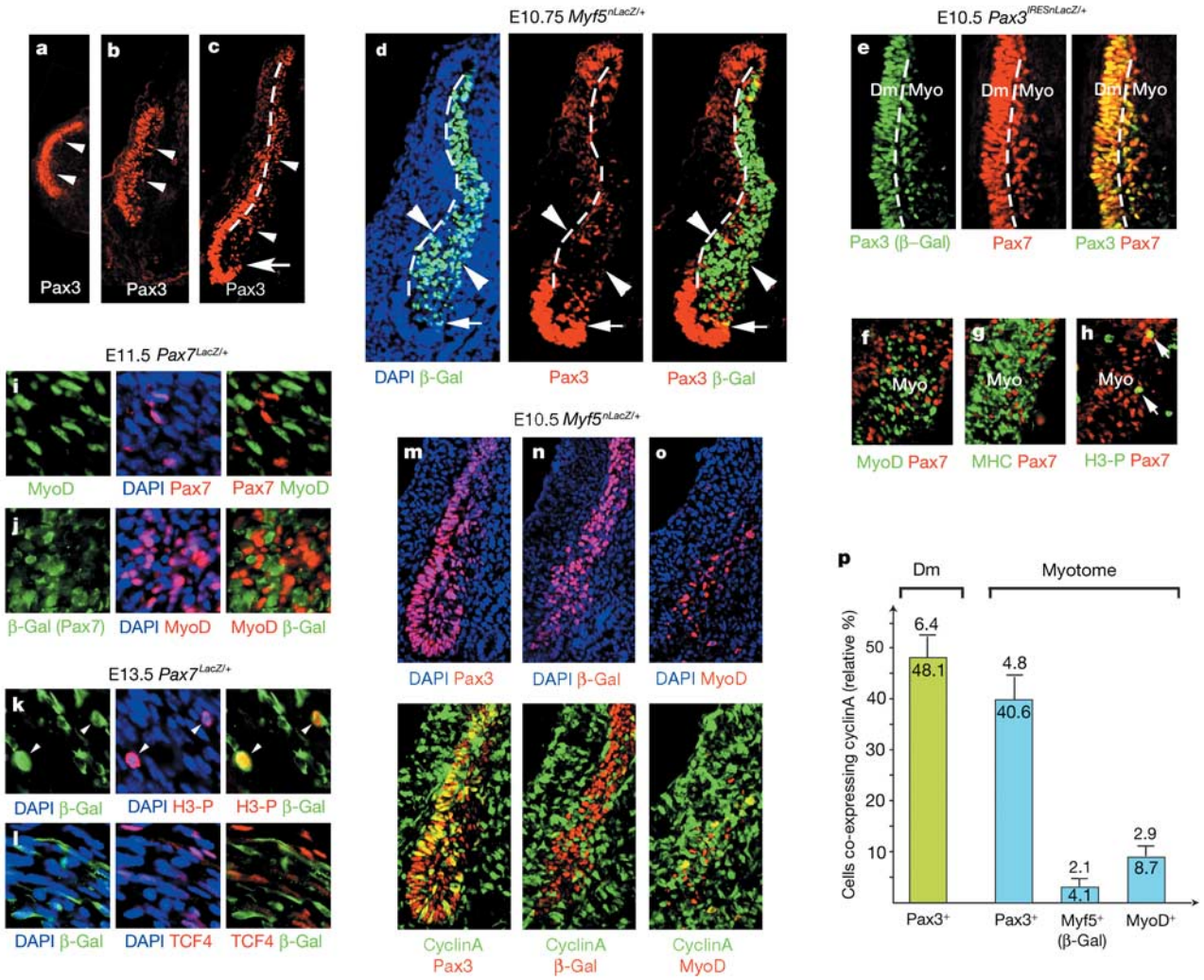

Figure 1 | Pax7 and Pax3 label a distinct cell population present in embryonic skeletal muscle. Where appropriate, detected proteins in Figs 1–4 are written underneath individual panels in the colour corresponding to the fluorescent label or stain. **a–c**, Transverse sections at different somite levels, stained with a Pax3-specific antibody. Arrowheads point to labelled cells in the myotome. In **c**, a dotted line shows the separation between the dermomyotome (on the left) and the myotome. At this stage, the dermomyotome is disintegrating in the central region while the epithelial structure is maintained hypaxially (arrow). **d**, Co-immunohistochemistry on transverse sections of thoracic somites (36-somite stage) from $Myf5^{nLacZ/+}$ embryos at E10.75, using antibodies recognizing Pax3 and β-gal from the $Myf5^{nLacZ/+}$ allele[24]. The myogenic markers do not co-localize with Pax3. Arrowheads indicate the width of the myotome. The arrow indicates the hypaxial extremity of the dermomyotome. A dotted line shows the separation between the dermomyotome (on the left) and the myotome. **e**, Co-immunohistochemistry on transverse sections of hypaxial thoracic somites from a $Pax3^{IRESnLacZ/+}$ embryo at E10.5, using antibodies recognizing β-gal and Pax7, showing co-localization in the myotome. Dm, dermomyotome, Myo, myotome. **f–h**, Co-immunohistochemistry on transverse sections of hypaxial thoracic somites from embryos at E10.5, using antibodies recognizing Pax7 and MyoD (**f**), MyHC (MHC) (**g**) or phosphorylated histone H3 (H3-P, **h**), showing that Pax7$^+$ cells are dividing. **i–l**, Co-immunohistochemistry on transverse sections of E11.5 shoulder muscle (**i**, **j**) and hypaxial trunk muscle (**k**, **l**) from $Pax7^{LacZ/+}$ embryos at E13.5 using antibodies directed against MyoD and Pax7 (**i**), MyoD and β-gal (**j**), H3-P and β-gal (**k**), and TCF4 and β-gal (**l**), showing that Pax7$^+$ cells are dividing and independent of connective tissue. **m–o**, Co-immunohistochemistry on transverse sections of trunk somites from $Myf5^{nLacZ/+}$ embryos at E10.5 using antibodies recognizing Pax3 (**m**), β-gal (**n**), MyoD (**o**) and cyclin A (bottom panels in **m–o**). DAPI staining is shown in the top panels of **m–o**. The hypaxial region is shown with Pax3-positive cells expressing cyclin A, whereas this is the case for only a few Myf5(β-gal)$^+$ or MyoD$^+$ cells. **p**, Quantification of ≥6 sections from at least two different embryos as shown in **m–o**. Error bars indicate standard deviation.

These observations strongly suggest that the dividing Pax3$^+$Pax7$^+$ cells provide a major source of myogenic cells for the formation of skeletal muscle fibres. When the *Pax3*$^{GFP/+}$ line is crossed with *Myf5*$^{nlacZ/+}$ mice, all β-gal$^+$ cells are also GFP$^+$ (Fig. 2p–r). Identical results were obtained with GFP and MyoD (Supplementary Fig. S1g–i and data not shown).

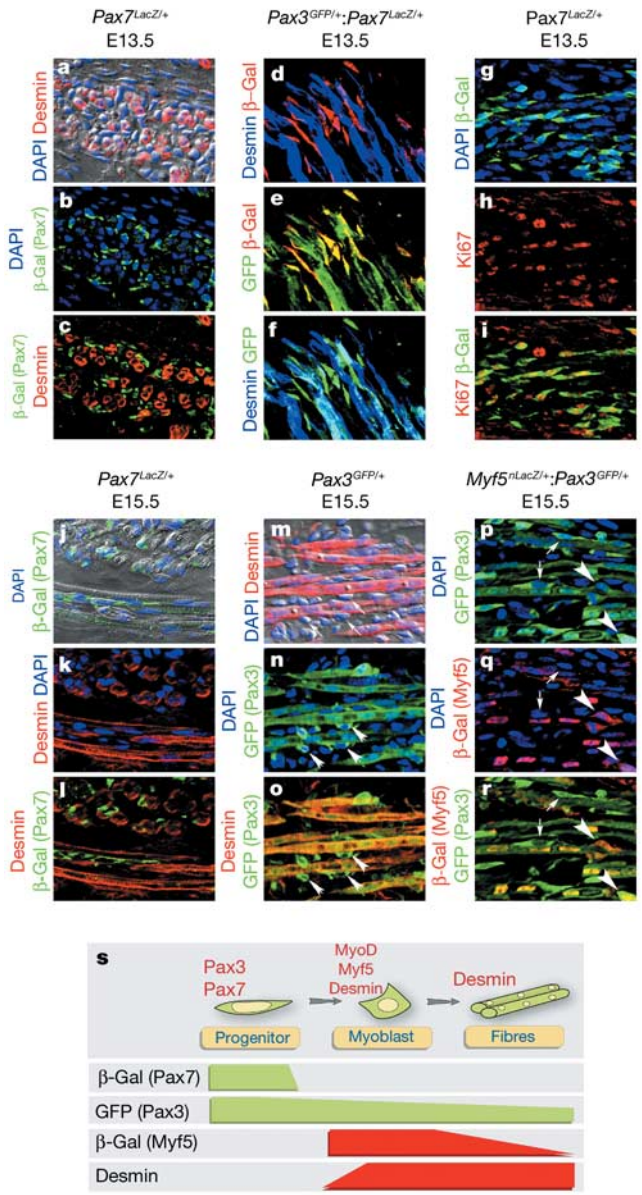

Figure 2 | Pax3$^+$Pax7$^+$ cells contribute to skeletal muscle. **a–i**, Co-immunohistochemistry at E13.5 on transverse sections from hypaxial trunk muscle of *Pax7*$^{LacZ/+}$ (**a–c**, **g–i**) or *Pax3*$^{GFP/+}$:*Pax7*$^{LacZ/+}$ (**d–f**) embryos, using antibodies directed against desmin (**a**, **c**, **d**, **f**), β-gal (**b–e**, **g**, **i**), or Ki67 (**h**, **i**). Phase-contrast image shown in **a**, GFP fluorescence in **e**, **f** and DAPI staining in **a**, **b**, **g**. **j–r**, Co-immunohistochemistry at E15.5 on transverse sections from hypaxial trunk muscle of *Pax7*$^{LacZ/+}$ (**j–l**), *Pax3*$^{GFP/+}$ (**m–o**) or *Myf5*$^{nLacZ/+}$:*Pax3*$^{GFP/+}$ (**p–r**) embryos, using antibodies directed against desmin (**k–m**, **o**), β-gal (**j**, **l**, **q**, **r**) or GFP (**n–p**, **r**). Phase-contrast images are shown in **j**, **m**, and DAPI staining is shown in **j**, **k**, **m**, **n**, **p**, **q**. GFP$^+$desmin$^-$ cells and GFP$^+$β-gal$^-$ cells are indicated by white arrows in **m–r**. GFP$^+$β-gal$^+$ myogenic cells are shown with arrowheads in **p–r**. **s**, Schematic showing the endogenous expression of Pax3 and Pax7 in resident muscle progenitor cells, the expression of Myf5 and MyoD in muscle myoblasts, and desmin in myoblasts and muscle fibres. This expression is compared with the relative expression of the LacZ, GFP and nLacZ reporter proteins generated from, *Pax7*^{LacZ}, *Pax3*^{GFP} and *Myf5*^{nLacZ} alleles, respectively.

We then investigated whether our findings on trunk muscles could be generalized to the limb, where the myogenic cells are derived from a progenitor cell population that migrates from the somites[14,15]. These cells express Pax3 but not Pax7, which is upregulated at E11 in the mouse embryo[9]. At E13.5, we found that cells expressing *Pax7*$^{LacZ/+}$ are distinct from the desmin-positive muscle cells of the limb (Supplementary Fig. S1c–f). Analysis of *Pax3*$^{GFP/+}$:*Pax7*$^{LacZ/+}$ embryos at this stage also showed that all the Pax7$^+$ cells express GFP (Supplementary Fig. S1d–f) and that all the MyoD$^+$ myogenic cells, which do not express Pax7, are marked by GFP (Supplementary Fig. S1g–l). We conclude that the Pax3$^+$Pax7$^+$ cells constitute a novel compartment of resident muscle progenitor cells that contribute to muscle growth during development both in the limbs and trunk.

By E15.5, cells that are Pax3$^+$ but Myf5(β-gal)$^-$ are located along, and in close contact with, the muscle fibres (Fig. 2p–r). Between E16.5 and E18.5 a basal lamina, marked by laminin expression, forms around the muscle fibres, including the associated Pax3$^+$Pax7$^+$ cells (Fig. 3a–o and Supplementary Fig. S1m–o). This location, under the basal lamina and in close proximity to the muscle fibre, is characteristic of satellite cells, which are the progenitor cells of postnatal skeletal muscle[21] and have been shown to originate from the somite in the chick embryo[22]. In the mouse, these cells express Pax7 (ref. 10), and in a subset of skeletal muscles also express Pax3 (F.R. and M.B., unpublished data). We found that *Pax3*$^{GFP/+}$ cells become embedded under the basal lamina by E18.5 (Fig. 3m–o and Supplementary Fig. S1m–o) and are still detectable during postnatal growth (Fig. 3p–r). We suggest that the resident muscle progenitor cells present in embryonic and fetal muscle later constitute the satellite cell

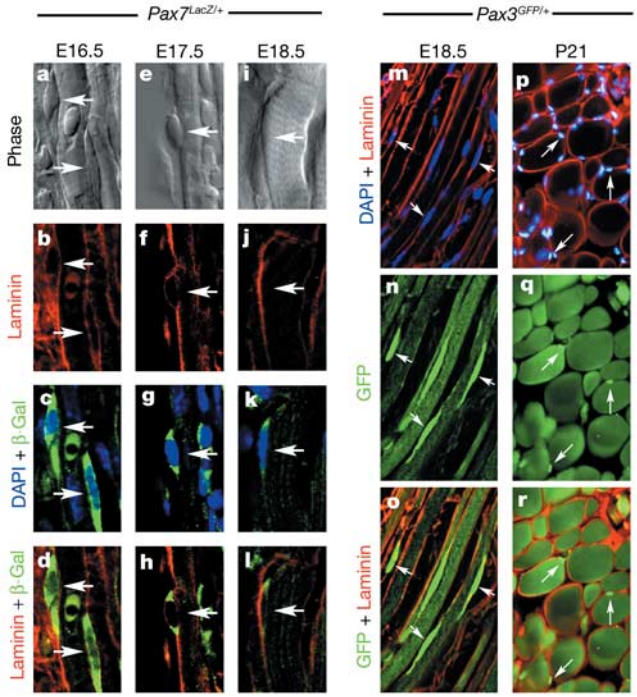

Figure 3 | Pax3$^+$Pax7$^+$ progenitors adopt a satellite cell position from late fetal stages. **a–l**, Co-immunohistochemistry on hypaxial trunk muscle from *Pax7*$^{LacZ/+}$ fetuses at E16.5 (**a–d**), E17.5 (**e–h**) and E18.5 (**i–l**), using DAPI staining (**c**, **g**, **k**) and antibodies directed against laminin (**b**, **d**, **f**, **h**, **j**, **l**) and β-gal (**c**, **d**, **g**, **h**, **k**, **l**). Phase-contrast images shown in **a**, **e**, **i**. β-gal$^+$ cells become embedded in the basal lamina and adopt satellite cell positions (indicated with white arrows). **m–r**, Co-immunohistochemistry on hypaxial trunk muscle from *Pax3*$^{GFP/+}$ embryos at E18.5 (**m–o**) or three-week-old (P21) mice (**p–r**) using DAPI staining (**m**, **p**) and antibodies directed against laminin (**m**, **o**, **p**, **r**) and GFP (**n**, **o**, **q**, **r**). White arrows indicate GFP-positive cells located in a satellite cell position.

population responsible for postnatal muscle growth and regeneration.

As previously reported, *Pax7* mutants show no overt muscle defect during development[9,18]. In contrast, in the trunk, *Pax3* mutant embryos display somite truncations[20] with loss of the hypaxial dermomyotome (Fig. 4b,e), resulting in the reduction and disorganization of hypaxial trunk musculature[3] (Fig. 4h, arrowhead). The somites of *Pax3*/*Pax7* double mutants are similar to those of *Pax3* mutants until E10.5 (Fig. 4b–c), with initial formation of skeletal muscle in the myotome (Suplementary Fig. S3a, b) under the control of the myogenic regulatory factors Myf5 (ref. 6) and/or Mrf4 (ref. 7). However, as development proceeds, somites become more severely affected and loss of $Pax3^{+}Pax7^{+}$ cells is observed (Fig. 4f). Subsequently, most skeletal muscles of the trunk, such as those of the overlying body wall (Fig. 4i), are severely compromised. By E13.5, only a few muscle fibres are detectable (Fig. 4l), and at E11.5 there is already a marked deficit of differentiated muscle cells (Fig. 4o). Pax3 (and Pax7 when substituted for Pax3, ref. 9) assures the survival of early myogenic progenitor cells in the hypaxial dermomyotome[4]. By E11.5, no further apoptosis is observed in the *Pax3* mutant, whereas in the *Pax3*/*Pax7* double mutant cell death still occurs (lower panels, Fig. 4m–o).

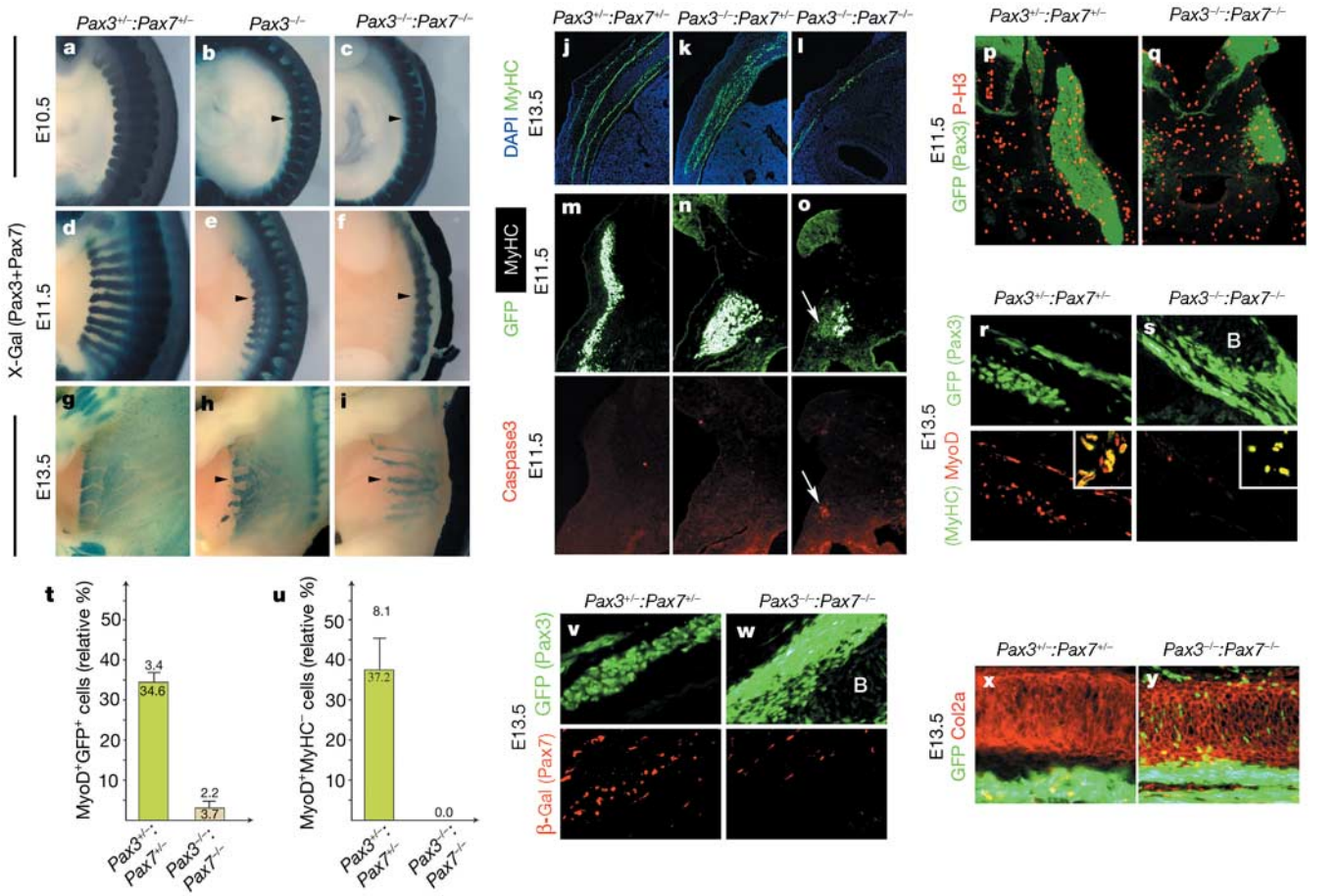

Figure 4 | Major myogenic defects in *Pax3/Pax7* double mutant embryos. **a–i**, X-gal staining of thoracic somites at E10.5 (**a–c**) and E11.5 (**d–f**) and of thoracic muscles at E13.5 (**g–i**) of $Pax3^{nLacZ/+}$:$Pax7^{LacZ/+}$ ($Pax3^{+/-}$:$Pax7^{+/-}$, **a**, **d**, **g**), $Pax3^{nLacZ/nLacZ}$ ($Pax3^{-/-}$, **b**, **e**, **h**) or $Pax3^{nLacZ/nLacZ}$:$Pax7^{LacZ/LacZ}$ ($Pax3^{-/-}$:$Pax7^{-/-}$, **c**, **f**, **i**) embryos. Arrowheads indicate somite and trunk muscle defects. **j–l**, Immunohistochemistry on transverse sections of body wall muscle of $Pax3^{GFP/+}$:$Pax7^{LacZ/+}$ ($Pax3^{+/-}$:$Pax7^{+/-}$, **j**), $Pax3^{GFP/GFP}$:$Pax7^{LacZ/+}$ ($Pax3^{-/-}$:$Pax7^{+/-}$, **k**) or $Pax3^{GFP/GFP}$:$Pax7^{LacZ/LacZ}$ ($Pax3^{-/-}$:$Pax7^{-/-}$, **l**) embryos at E13.5, using DAPI staining and an antibody against MyHC. Loss of skeletal muscle is seen in the double mutant. **m–o**, Co-immunohistochemistry on transverse sections of thoracic somites of E11.5 embryos as in **j–l**, using antibodies recognizing MyHC (top panels) and the activated form of caspase 3, which labels apoptotic cells (bottom panels). Endogenous GFP fluorescence is detectable above background and co-localizes with active caspase-3 (arrow in **o**), indicating that, in contrast to the *Pax3* mutant embryos, apoptosis is maintained in the *Pax3/Pax7* double mutants at E11.5. **p–y**, Co-immunohistochemistry (**p–s**, **v–y**) and histograms (**t**, **u**) using $Pax3^{GFP/+}$:$Pax7^{LacZ/+}$ ($Pax3^{+/-}$:$Pax7^{+/-}$) or $Pax3^{GFP/GFP}$:$Pax7^{LacZ/LacZ}$ ($Pax3^{-/-}$:$Pax7^{-/-}$) embryos. **p**, **q**, Transverse sections of thoracic somites from E11.5 embryos using antibodies recognizing GFP and phosphorylated histone H3 (P-H3), which labels mitotic cells. No proliferation defects are observed in the double mutant embryos. **r**, **s**, Transverse sections of embryos at E13.5 using antibodies recognizing GFP (top panels), MyoD (bottom panels) and MyHC (insets in bottom panels). Insets show the co-localization (in yellow) of MyoD and MyHC in the control and *Pax3/Pax7* double mutants. There are no undifferentiated $MyoD^{+}/MyHC^{-}$ cells in the double mutants. **t**, Quantification of the number of GFP^{+} cells co-expressing MyoD in embryos shown in **r**, **s** (≥6 sections from 2 independent embryos in each case). **u**, Quantification of the number of $MyoD^{+}$ cells co-expressing MyHC. In contrast to the control embryos, in which 37% of the MyoD + cells are not terminally differentiated, all the $MyoD^{+}$ cells co-express the differentiation marker MyHC in the double mutant embryos, demonstrating that at this stage no $MyoD^{+}$ myogenic cells are contributing to muscle growth. Standard deviations are indicated on the histograms. **v**, **w**, Transverse sections of E13.5 embryos at the level of the rib, which is normally adjacent to intercostal muscle, using antibodies recognizing GFP (top panels) and β-gal (bottom panels), showing that only cells marked with the stable GFP reporter are still present. In **w** (top panel), some GFP^{+} cells localize in the bone (B) of double mutant embryos. **x**, **y**, Transverse sections in E13.5 embryos at the level of the ribs, normally in proximity to hypaxial muscles, using antibodies recognizing GFP and collagen2a (Col2a) which marks the condensing cartilage of the ribs. In the double mutant, GFP^{+} cells are now located within and in the immediate vicinity of the rib.

Cell proliferation, another factor that may affect the capacity of $Pax3^{+}Pax7^{+}$ progenitor cells to contribute to muscle growth, does not appear to be affected in the GFP^{+} population in $Pax3^{GFP/GFP}$:$Pax7^{LacZ/LacZ}$ mutant embryos (Fig. 4p, q). Notably, GFP-positive cells are still present at E13.5, when there is a marked deficit in skeletal muscle. They are mainly $MyoD^{-}$ (Fig. 4r, s, lower panels), and the remaining $MyoD^{+}$ cells (3.7% of GFP-expressing cells, compared with 34.6% in the presence of Pax3 and/or Pax7, Fig. 4t) are all co-expressing terminal differentiation markers (Fig. 4r, s, lower panels, u). They probably correspond to differentiated cells dating from the first phase of embryonic myogenesis, when the myotome forms in the absence of Pax3 and Pax7 (Supplementary Fig. S3). This indicates that in the absence of both Pax proteins, myogenic progenitor cells at later embryonic and fetal stages are not specified as skeletal muscle. Instead, they downregulate transcription of the *Pax* genes, as shown in Fig. 4v, w, lower panels, where β-gal from the $Pax7^{LacZ}$ allele is no longer detectable (3.2% versus 37.7%) in cells in which the more stable GFP reporter produced from the $Pax3^{GFP}$ allele (Fig. 4s, Fig. 2s and Supplementary Fig. S2) is still present. Identical results to those shown for $Pax7^{LacZ}$ were obtained with a $Pax3^{IRESnLacZ}$ allele (data not shown). The GFP-positive mutant cells probably assume non-myogenic fates, as shown by their presence in bones, where they are associated with the expression of markers such as collagen 2a (Col2a, Fig. 4y), which is characteristic of cartilage cells.

Our results show that after the initial formation of the myotome, which has been a focus in the study of myogenic regulation[2,16], subsequent embryonic myogenesis depends on the expression of Pax3 and Pax7, making these factors key upstream regulators of the myogenic process. In the absence of both Pax proteins, resident muscle progenitor cells do not enter the myogenic programme. Double mutants die at mid-gestation, but given the persistence of this cell population and its demonstrated contribution to fetal muscles, we conclude that the skeletal musculature of the fetus also depends on Pax3 and Pax7. The apparent acquisition of satellite cell properties by the $Pax3^{+}Pax7^{+}$ cells in late fetal muscle suggests that the progenitor cells of postnatal and adult muscle also derive from this population. These cells are compromised in the *Pax7* mutant[10], but some satellite cells are still detectable, suggesting that they are specified correctly[11]; furthermore, there is no deficit in satellite cells immediately postnatally (F.R., D. Montarras and M.B., unpublished data). This is consistent with a requirement for either Pax3 or Pax7 to generate the cell pool of muscle progenitors from which satellite cells derive. Complementary observations on the origin of the $Pax3^{+}Pax7^{+}$ population from the dermomyotome of the chick embryo using long-term lineage tracing[23] suggest that the progenitor cells of fetal and postnatal muscle derive from the paraxial mesoderm of the embryonic somite.

The resident muscle progenitor cells that we have identified constitute the principal proliferative cell population of developing skeletal muscle. It is important to determine whether these cells self-renew as a true stem cell population (see Figs 1m–p and 2g–i). Pax3 and Pax7 have an important role in conferring myogenic potential on these progenitor cells, thus assuring the major phases of skeletal muscle formation as the organism develops. Such cells represent a potentially exploitable resource in the context of cell therapy for muscle diseases.

METHODS

Mice. Generation and genotyping of the $Pax3^{nLacZ/+}$, $Pax3^{IRESnLacZ/+}$ and $Pax7^{LacZ/+}$ alleles have been previously described[9,18,20]. The $Pax3^{GFP/+}$ allele will be described elsewere in detail. Briefly, enhanced (E)GFP replaces the *Pax3* coding sequence of exon 1, as previously reported for DsRed in the $Pax3^{DsRed/+}$ allele[9].

X-Gal staining, histology, immunohistochemistry and *in situ* hybridization. X-Gal staining, histology, immunohistochemistry and whole-mount *in situ* hybridization were performed as previously reported[9]. Antibodies used were as follows: MyoD, either a rabbit polyclonal (Santa Cruz; 1:200 dilution) or a mouse monoclonal antibody (clone 5.8A, DAKO; 1:200); desmin, mouse monoclonal antibody (clone D33, DAKO; 1:200); laminin, either a mouse monoclonal (clone 4C7, DAKO; 1:100) or a rabbit polyclonal antibody (Sigma; 1:200); Pax7, mouse monoclonal antibody (Developmental Studies Hybridoma Bank; 1:100); Pax3, mouse monoclonal antibody (provided by M. Bronner-Fraser; 1:100); β-gal, either a rabbit polyconal (provided by J.-F. Nicolas; 1:500) or a mouse monoclonal antibody (clone Gal13, SIGMA; 1:100); phospho-histone H3, mouse monoclonal antibody (Cell Signalling; 1:200); TCF4, chicken polyclonal antibody (provided by G.R. Dressler; 1:200); MyHC, rabbit polyclonal antibody (provided by G. Cossu; 1:250); GFP, mouse monoclonal antibody (Biovalley; 1:1,000); Col2a, mouse monoclonal antibody (abcam; 1:100); Ki67, rabbit polyclonal antibody (Pharmingen; 1:200); cyclin A, rabbit polyclonal antibody (provided by A. Fernandez; 1:200).

Secondary antibodies were coupled to a fluorochrome, Alexa 350, 488, 546, 594 or 647 (Molecular Probes), used at 1:250 (Alexa 350 and 488) or 1:1,500 (Alexa 546, 594 or 647) dilutions. Images are obtained with Apotome Zeiss and Axiovision software. This system provides an optical section view reconstructed from fluorescent samples, using a series of 'grid projection' (or 'structured illumination') acquisitions. Figures were assembled using the Photoshop CS application (Adobe) and a PowerMacG4. Percentage figures given in the text on antibody labelling were based on the analysis of ≥6 sections from at least two embryos.

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