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Lineage restriction of the myogenic conversion factor *myf-5* in the brain

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SUMMARY

***myf-5* is one of four transcription factors belonging to the *MyoD* family that play key roles in skeletal muscle determination and differentiation. We have shown earlier by gene targeting *nlacZ* into the murine *myf-5* locus that *myf-5* expression in the developing mouse embryo is closely associated with the restriction of precursor muscle cells to the myogenic lineage. We now identify unexpected expression of this myogenic factor in subdomains of the brain. *myf-5* expression begins to be detected at embryonic day 8 (E8) in the mesencephalon and coincides with the appearance of the first differentiated neurons; expression in the secondary prosencephalon initiates at E10 and is confined to the ventral domain of prosomere p4, later becoming restricted to the posterior hypothalamus. This expression is observed throughout embryogenesis. No other member of the *MyoD* family is detected in these regions, consistent with the lack of myogenic conversion. Furthermore, embryonic stem cells expressing the *myf-5/nlacZ* allele yield both skeletal muscle and neuronal cells when differentiated in vitro. These observations raise questions about the role of *myf-5* in neurogenesis as well as myogenesis, and introduce a new lineage marker for the developing brain.**

Key words: myogenic conversion, myf-5, homologous recombination, brain development

INTRODUCTION

A prerequisite for the establishment of tissue-specific phenotypes is a progressive loss in stem cell multipotentiality leading to more restricted cell lineages. A group of transcription factors belonging to the basic-Helix-Loop-Helix (bHLH) superfamily: MyoD (Davis et al., 1987), myogenin (Edmondson and Olson, 1989; Wright et al., 1989), myf-5 (Braun et al., 1989) and MRF4 (Rhodes and Konieczny, 1989) (also identified as herculin (Miner and Wold, 1990) or myf-6 (Braun et al., 1990)) has been implicated in this process for skeletal muscle. In addition to activating many muscle-specific genes, these myogenic regulatory factors, when overexpressed in cultured non-muscle cells, will effect their conversion to myogenesis (Weintraub et al., 1991). This phenomenon led to the proposal that this family of factors plays a key role in skeletal muscle determination as well as differentiation. Consistent with a role in muscle specification in vertebrates, cell culture experiments and in situ hybridisation of embryos led to the conclusion that the expression of these myogenic factors is restricted to cells of the skeletal muscle lineage (see Buckingham, 1992; Olson and Klein, 1994). The functional importance of these factors in myogenesis is demonstrated by the fact that, in the absence of myf-5 and MyoD, mice lack skeletal muscle (Rudnicki et al., 1993) while, in the absence of myogenin, they have myoblasts but lack most differentiated muscle fibers (Hasty et al., 1993; Nabeshima et al., 1993). Since myf-5 is the only member of the MyoD family present in precursor muscle cells before muscle formation in the mouse (Ott et al., 1991; Buckingham, 1992), we chose to use this gene as a cell lineage marker to identify precursor muscle populations after gastrulation. In order to follow the myf-5 expressing cell population in normal (*myf-5*$^{+/-}$) and mutant (*myf-5*$^{-/-}$) mice, we have targeted the *myf-5* locus in embryonic stem (ES) cells with the introduction of *nlacZ* into the first exon of the gene and generated mice in which *nlacZ* expression is controlled by the *myf-5* regulatory sequences (Tajbakhsh and Buckingham, 1994). We report here the unexpected finding that *myf-5*, but not the other myogenic factors, is expressed in defined subdomains of the developing brain, from the earliest stages of neuronal cell differentiation.

MATERIALS AND METHODS

Gene targeting in ES cells and embryo manipulations

For homologous recombination, the *nlacZ* gene (*lacZ* with an associated nuclear localisation signal) was fused to the first 13 amino acids in exon 1 of the *myf-5* gene. The vector containing 5.5 kbp and 1.1 kbp of the *myf-5* locus flanking the *nlacZ* and *neomycin*-resistance genes was used to target D3 (courtesy of R. Kemler) or HM1 (courtesy of David Melton) ES cells. Targeted ES clones were verified by Southern analysis and subsequently used to generate chimaeric embryos by injections into precompaction morula. After recompaction, manipulated morulae were implanted into oviducts of foster mothers that were plugged the same day; the day of implantation was considered to be E0.5. Details of the targeting and analysis of β-galactosidase (β-gal) expression patterns are given elsewhere (Tajbakhsh and Buckingham, 1994; S. Tajbakhsh, E. Bober, C. Babinet, S. Pournin, H. Arnold, and M. Buckingham, unpublished data). Results shown have been reproduced with several independently targeted ES clones, two DNA constructs containing different *neomycin* reporter genes, and heterozygote embryos. *nlacZ* expression during skeletal

myogenesis, in mice generated from these ES cells (Buckingham and Tajbakhsh, 1993; Tajbakhsh and Buckingham, 1994), corresponded to observations made by in situ hybridisation (Ott et al., 1991, Buckingham, 1992).

ES cells containing *nlacZ* in one allele of the *myf-5* gene were differentiated in vitro essentially as described (Robertson, 1987) to give skeletal muscle and neuronal cells.

Immunohistochemistry

For cryostat sections, embryos were fixed in fresh 4% paraformaldehyde (E12.5, 1.5 hours), washed in phosphate-buffered saline (PBS), transferred to 15% sucrose, then 15% sucrose/7% gelatin for sectioning. 10 μm cryostat sections were rinsed in PBS and permeabilized in PBS/0.05% Triton X-100 for 20 minutes. After 3× 5 minutes washes in PBS, sections were incubated in PBS/1% BSA/1% goat serum for 10 minutes to reduce nonspecific binding, followed by incubation with the primary antibody(s). After 3× 5 minute washes in PBS, anti-βIII tubulin monoclonal (Sigma, 1:100 dilution), and anti-β-galactosidase polyclonal (1:200), or anti-tyrosine hydroxylase polyclonal (Institut Jacques Boy, 1:1500), or anti-Troponin T (Amersham, 1:100) primary antibodies were used for 1 hour incubations. For immunofluroescence, sections were incubated with fluorescein-conjugated goat anti-rabbit IgG and rhodamine-conjugated goat anti-mouse IgG for 30 minutes (Tajbakhsh et al., 1994). For peroxidase staining, a donkey anti-rabbit biotinylated secondary IgG (Amersham, 1:200) was incubated for 1 hour, followed by PBS washes as above, and incubation with ExtrAvidin peroxidase (Sigma, 1:20) for 30 minutes at 37°C. Detection of peroxidase activity was done with 3-amino-9-ethyl-carbazole (AEC, Sigma) according to the manufacturer's recommendations. Antibody incubations were carried out at room temperature. Anti-βIII tubulin does not react with skeletal muscle cells (Tajbakhsh et al., 1994; present study). Sections and cultures were mounted in 75% glycerol PBS (pH 8) and observed under an epifluorescence Zeiss Axiophot microscope. For better morphological detail, some embryos were embedded in Historesin (Jung) according to the manufacturer and 4 μm sections were made.

Cloning of myf-5 cDNA and in situ hybridisations

The murine myf-5 cDNA was synthesized with RNA isolated from the C2/7 skeletal muscle cell line (courtesy of D. Montarras and C. Pinset) by reverse-transcriptase PCR (primers: 5′-GGA ATT CTA GAG GAT CCA GAG AGA CAG TCC CAA CTC CGG GA-3′ and 5′-ATC GAT GGT ACC GGA ATT CCT GGC TCA TGA TTG GGC AAA GTA CTGT-3′) using 2 μg myoblast RNA, the reverse primer and superscript reverse transcriptase (Gibco, BRL) under the manufacturer's conditions. PCR amplification was carried out with 1/2 of the cDNA material, after denaturation at 96°C for 8 minutes with 500 ng of each primer, in the presence of 200 μM dNTP using PFU Taq polymerase (Stratagene) for 20 cycles as follows: 93°C, 30 seconds, 55°C, 30 seconds and 72°C, 4.5 minutes. After restriction digestion and fragment isolation, the cDNA was cloned as a *Bam*HI/*Eco*RI fragment in Bluescript (Stratagene). Antisense RNA (1.2 kb), corresponding to the 3′ end of the transcript was generated using the T7 promoter after linearization of the plasmid with *Sma*I. The probe was hydrolysed for 40 minutes. Antisense riboprobes for myf-5, together with myogenin and MyoD (Sassoon et al., 1989), were used for in situ hybridisations as described (Sassoon and Rosenthal, 1993).

RESULTS

During the initial stages of brain development, the cephalic region of the neural tube undergoes divisions to form the hindbrain, midbrain and forebrain. Recent experiments have suggested that the forebrain can be subdivided into segments that may be defined by gene-specific expression domains (Bulfone et al., 1993; Figdor and Stern, 1993), as described for the hindbrain (Krumlauf, 1994). In particular, the forebrain which comprises the diencephalon (p1-p3) and secondary prosencephalon (p4-p6), has been proposed to consist of six prosomeres (p1 to p6; see Puelles and Rubenstein, 1993). *myf-5* expression in neural tissue was first observed in embryos at E8 (about 10 somites) and by late E8.5 β-gal$^+$ cells are present in the prospective mesencephalic/diencephalic boundary region (Fig. 1A). These cells first appear dispersed, initially centered around the midbrain; by E10.5, this expression becomes confined to the basal zone of the midbrain/forebrain boundary in the region of the cranial flexure and does not span the midline (Figs 1B, 2A-C). We tentatively identify the sharp rostral limit of this expression domain at the p2/p3 boundary (see Fig. 2B).

A second region slightly rostral to this area, in the secondary prosencephalon (p4), also contains β-gal$^+$ cells at E10.5. By E11.5, both of these sites express *myf-5* prominently (see Fig.

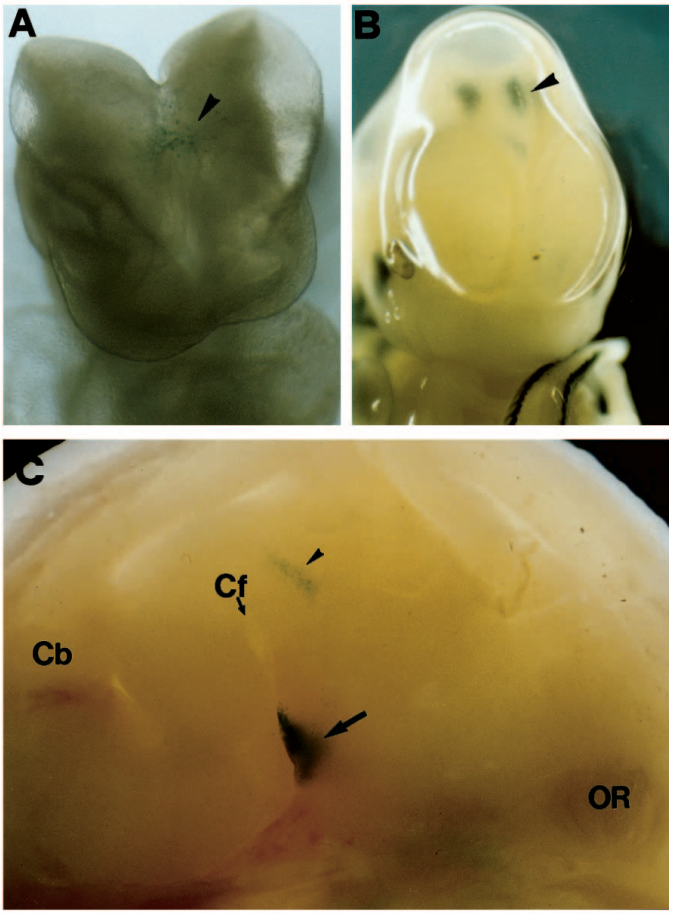

Fig. 1. Regionalised expression of *myf-5* in the mouse brain. (A) Frontal view of a late E8.5 embryo at the open neural head fold stage showing β-gal$^+$ cells in the prospective mesencephalic/diencephalic boundary region (arrowhead). (B) Frontal view of E11 embryo showing regionalised *myf-5* expression in the cranial flexure of the mesencephalic/diencephalic boundary (arrowhead). This expression domain does not span the midline. (C) Whole-mount view of E16.5 embryo, sectioned through the hypothalamus, showing localised *myf-5* expression in this region (arrow). A second site of expression located in the basal zone of p1 (arrowhead) is also indicated. Cf, cranial flexure; Cb, cerebellum; OR, optic recess.

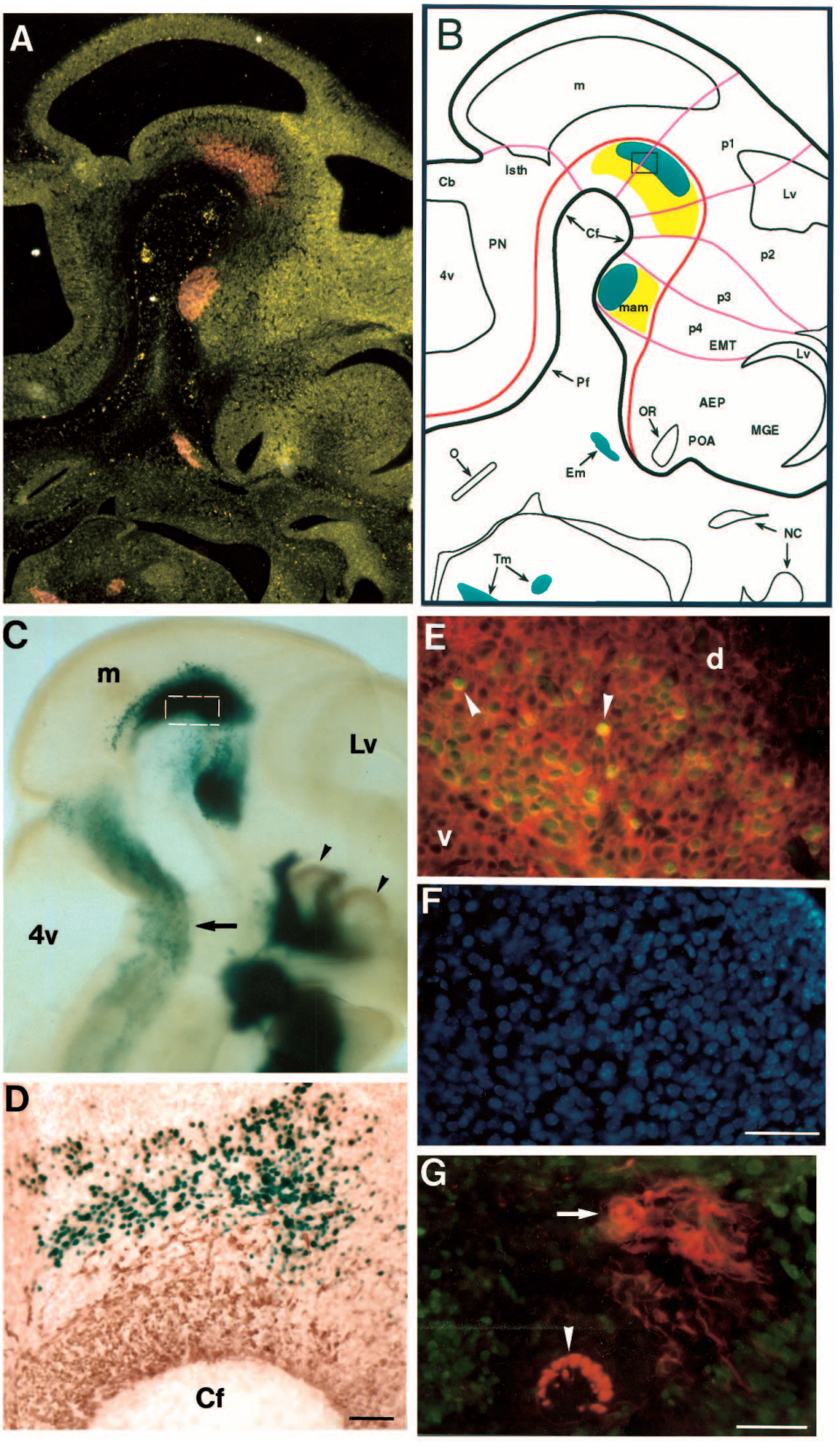

Fig. 2. *myf-5* is expressed in βIII tubulin-positive neurons in the brain.
(A) Paramedial resin section of an E12.5 embryo, taken with dark-field optics; β-galactosidase staining appears pink.
(B) Schema of the section shown in A according to the hypothetical neuromeric model described (see Puelles and Rubenstein, 1993). The orange line delineates the sulcus limitans representing the alar and basal plate divisions. Divisions of mesencephalon and prosomeres 1-4 are indicated with violet lines. Sites of *myf-5* expression corresponding to the section in A are indicated in blue. Yellow zones delimit the overall zone of *myf-5* expression in the brain as seen by whole mount. (C) Lateral view of an E11.5 heterozygote embryo showing continuous β-gal$^+$ cells in the spinal cord extending through the pontine flexure (arrow) and rhombencephalon and terminating at the caudal boundary of the cerebellum. This view represents *myf-5* expression ventrally and on either side of the midline axis of the spinal cord (Tajbakhsh et al., 1994). The eyes (arrowheads) on either side of the embryo are partially encompassed by extraocular muscle masses. Muscle masses caudal to this region constitute the mandibular and tongue muscles. The embryo was stained with X-gal, dehydrated in 100% ethanol and rendered transparent using benzyl alcohol:benzyl benzoate (1:2).
(D) Heterozygote E12.5 cryostat section showing tyrosine hydroxylase (TH)-positive midbrain dopaminergic neurons (brown) do not colocalise with *myf-5* expressing cells (blue). The section was stained with X-gal (Tajbakhsh et al., 1994) then reacted with TH antibody (see Materials and Methods). This region corresponds to the white hatched area delineated in C. Note β-gal$^+$ cells appear dorsal and rostral to the dopaminergic neurons. (E) Colocalisations of β-gal and βIII tubulin in *myf-5* expressing cells in the m/p1 region of an E12.5 embryo. Cryostat sections reveal rhodamine labelled βIII tubulin in the cytoplasm and fluorescein labelled β-gal in the nucleus. Slight overlaps of the two signals into the nucleus and the cytoplasm, respectively allow the unambiguous identification of β-gal$^+$ cells within neuronal cells as seen by the yellow staining around nuclei (arrowheads). Only a subset of βIII tubulin-positive cells express β-gal. This region corresponds to the boxed area indicated in B. (F) Hoechst 33258 staining of nuclei of the section shown in E. (G) Control showing mutually exclusive labelling of βIII tubulin-positive neurons within (arrowhead, retinal ganglia) and around (arrow, ciliary ganglia) the eye epithelium, and β-gal$^+$ extraocular muscles (green). Bar = 50 μm. AEP, anterior entopeduncular area; Cb, cerebellum; Cf, cranial flexure; d, dorsal; Em, extraocular muscles; EMT, eminentia thalami; Isth, isthmus of mesencephalon; Lv, lateral ventricle; 4v, fourth ventricle; m, mesencephalon; mam, mammillary area in basal zone of p4; MGE, medial ganglionic eminence; NC, nasal cavity; O, otic capsule; OR, optic recess; p1-p4, prosomeres 1-4; Pf, pontine flexure; POA, anterior preoptic area; PN, pons; Tm, tongue muscles; v, ventral.

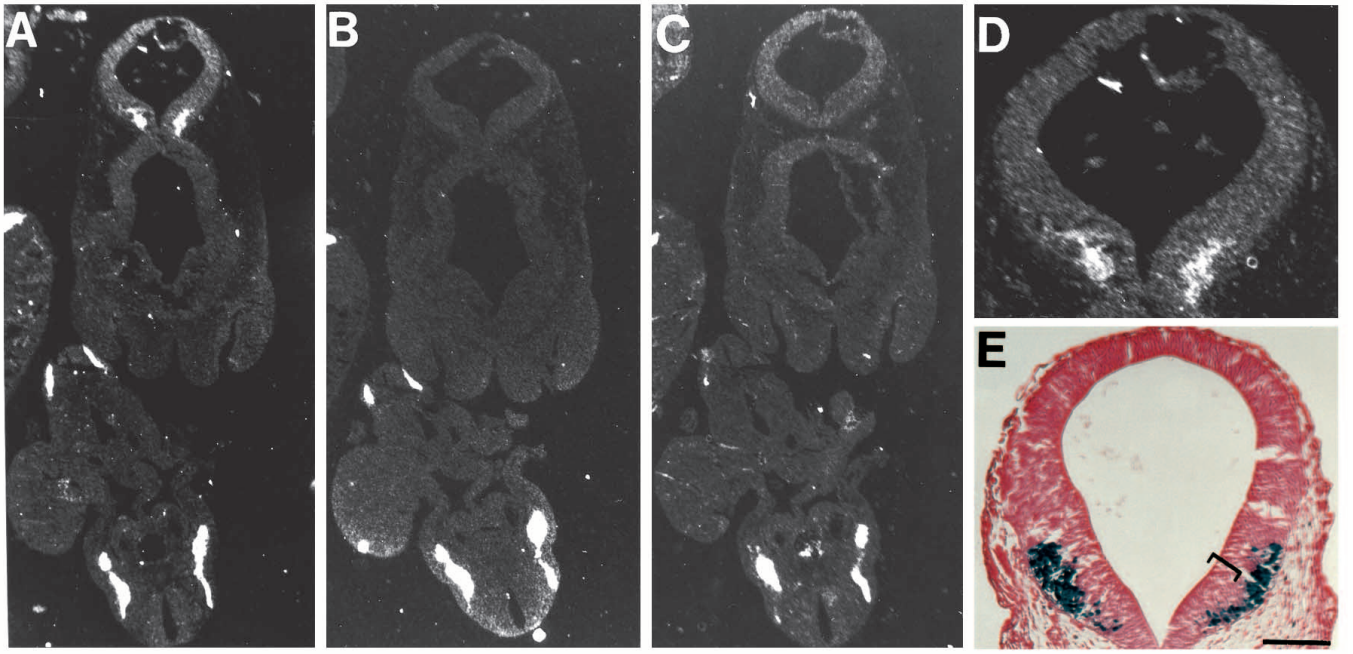

Fig. 3. myf-5 endogenous transcripts in the brain. In situ hybridisations of serial frontal sections from an E10.5 embryo, using myf-5 (A,D), myogenin (B) and MyoD (C) antisense riboprobes. Transcripts for all three genes are detected in the myotomes but only myf-5 transcripts appear in the brain. (D) Enlargement of mesencephalic region in A showing myf-5 transcripts confined to the basal layer of the neuroepithelium. (E) Frontal section of an E10.5 chimaeric embryo showing β-gal$^+$ cells in the basal layer of the neuroepithelium. The myf-5 signal in both cases appears in mantle layer cells. Cells in the ventricular zone (square bracket) are negative. Sections on either side of the *MyoD* and *myogenin* sections were hybridised with myf-5 antisense probes to ensure *myf-5* expression throughout this domain (data not

2C). *myf-5* expression in this region marks the ventral domain of p4 in the mammillary area of the prosencephalon (Fig. 2A-C). By E14.5 this labelling becomes restricted to the posterior hypothalamus. Fig. 1C shows a whole-mount view of an E16.5 fetal brain revealing *myf-5* expression in the mammillary body in the posterior hypothalamus. This site of expression continues to be present after birth (data not shown).

To determine whether *myf-5* expression is in neurons in the brain, we used βIII tubulin (Moody et al., 1987), a marker previously shown (Easter et al., 1993) to label the first differentiating neuronal cells of the neuroepithelium at E8.5, in the region of the midbrain where β-gal$^+$ cells are seen. Fig. 2E,F show an enlargement of a section from the midbrain/forebrain region of an E12.5 embryo (see Fig. 2B). βIII tubulin labelling and β-gal$^+$ cells colocalise in this region (Fig. 2E). A similar colocalisation was observed with β-gal and βIII tubulin in the *myf-5*-expressing cells located in p4 (data not shown). As expected, β-gal$^+$ cells in the muscles surrounding the eye are negative for βIII tubulin (Fig. 2G) confirming our earlier observation that this antibody does not cross react with skeletal muscle cells (Tajbakhsh et al., 1994). The onset of *myf-5* expression in the mesencephalon/p1 and its ventral location in the basal plate spanning the midbrain/forebrain junction led us to test whether this expression correlates with the birth of dopaminergic neurons in this area. However, antibody labelling for tyrosine hydroxylase, which is a marker for these neurons, did not reveal any colocalization with the β-gal$^+$ cells (Fig. 2D). The dopaminergic neurons are located ventrally and slightly caudally to the *myf-5* expressing cells. We recently reported the localised expression of *myf-5* in a subpopulation of cells in the mouse neural tube, in cells that express βIII tubulin, but not the skeletal muscle marker, myosin heavy chain (MyHC) (Tajbakhsh et al., 1994). Fig. 2C shows that this β-gal labelling extends the length of the spinal cord rostrally into the brain at E11.5 terminating in the most rostral domain of the rhombencephalon.

In order to confirm that *nlacZ* expression from the mutant *myf-5* allele reflects expression of the endogenous gene, we carried out in situ hybridisations on E10.5 embryos using an antisense myf-5 probe. Fig. 3A,D show that endogenous myf-5 transcripts in the mesencephalon colocalise with β-gal$^+$ cells (Fig. 3E), and that both are regionalised in the basal zone of the neuroepithelium. These cells located in the mantle layer express the differentiation marker βIII tubulin (Easter et al., 1993; data not shown). Unlike adjacent cells in the ventricular zone, mantle layer cells are no longer proliferating rapidly (Jacobson, 1978).

Despite significant levels of myf-5 transcripts in the forebrain, no muscle fibers nor transcripts encoding muscle proteins are detected. The question therefore remains: why do cells that express the *myf-5* gene not make muscle in vivo? In skeletal muscle, more than one myogenic factor is necessary for establishment of the full phenotype (see Olson and Klein, 1994); *myogenin*, particularly, is implicated in skeletal muscle differentiation (Hasty et al., 1993; Nabeshima et al., 1993). Therefore the expression status of the other members of the MyoD family in the brain was investigated. Fig. 3A-C show serial frontal sections of an E10.5 mouse embryo hybridised with myf-5, myogenin or MyoD antisense probes. While all three genes are expressed strongly in the myotomes, only myf-

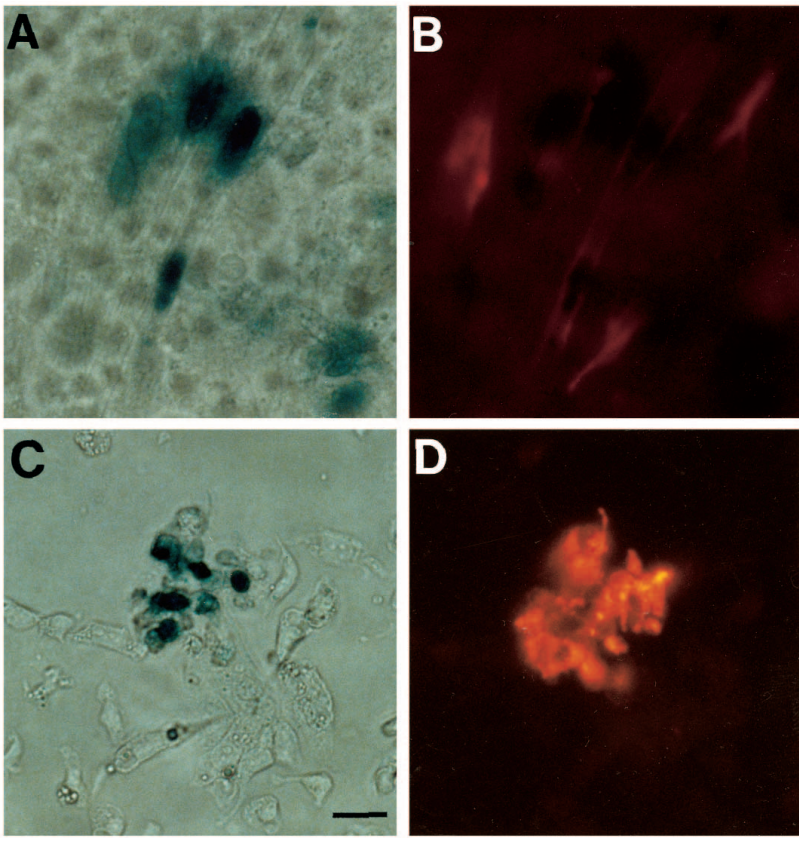

Fig. 4. *myf-5* is expressed in neuronal and skeletal muscle derivatives of differentiated ES cells. ES cells containing *nlacZ* in one allele of the *myf-5* gene were differentiated in vitro to give skeletal muscle and neuronal cells. (A) Phase-contrast of β-gal$^+$ myotubes; (B) the cells shown in A, reacted with the monoclonal antibody to TnT and a secondary antibody conjugated with rhodamine show colocalisation of these two markers; (C) phase contrast of β-gal$^+$ cells; (D) the cells in C were reacted with βIII tubulin monoclonal antibody and a secondary antibody conjugated with rhodamine; these two markers colocalise within the same cells. Bar, 50 μm.

5 transcripts are present in the embryonic brain. These results were confirmed with sagittal sections of E10.5 embryos (data not shown). *MRF4*, which is predominantly expressed in adult skeletal muscle, was not detected by RT-PCR of this region (data not shown).

Since *myf-5*-expressing cells in the brain also express a neuronal-specific marker, we were interested in examining the ES cells from which the myf-5/nlacZ mice were derived. Targeted ES cells carrying the *myf-5/nlacZ* allele when differentiated in vitro, yield β-gal$^+$ mononucleated cells and skeletal muscle myotubes. Whereas β-gal$^+$ myotubes were also positive for the muscle marker *troponin T* (*TnT*, Fig. 4A,B), some β-gal$^+$ mononucleated cells express the neuronal marker βIII tubulin (Fig. 4C,D). We conclude that these are *myf-5*-expressing neuronal cells, which in vivo contribute to the central nervous system.

DISCUSSION

This report provides the first evidence that a gene of the MyoD family is expressed in specific anatomical subdomains of the developing brain. This finding is unexpected given that, at least in vitro, the myogenic factors are capable of converting many cell types to muscle. Recent reports using transgenic mice have also demonstrated that ectopic over-expression of either *myf-5* or *MyoD* in the brain (Santerre et al., 1993) or heart (Santerre et al., 1993; Miner et al., 1992), respectively, leads to some conversion of these tissues to skeletal muscle fibers. In the case of the brain, the myf-5 transgene was under the control of a retroviral promoter; expression of the transgene was not reported in cells expressing the endogenous gene. No sufficiently specific antibodies are available to show unambiguously that myf-5 protein is expressed in the regions of the brain where the transcript of the endogenous gene is detected. However, in the targeted *myf-5* allele, β-gal synthesis is under the myf-5 mRNA leader sequence and ATG and, consequently, it is probable that the translation of β-gal mRNA reflects that of the myf-5 mRNA in these cells. It is therefore possible that the lack of myogenic conversion in these regions is related to a threshold level (see Gurdon et al., 1992) necessary to activate other myogenic factors such as myogenin, which is required for muscle differentiation. Alternatively, myogenic coactivators may be lacking in *myf-5*-expressing cells in the brain, or negative regulators may suppress the conversion properties of endogenously expressed myf-5. We would stress that the sites of *myf-5* expression in the brain do not reflect a transitory expression of the gene in a few dispersed cells, but rather continued expression concentrated in defined regions of the developing brain. The skeletal muscle transcription factor MEF2, like myf-5, can effect myogenic conversion (see Kaushal et al., 1994). This family of factors is also expressed in the CNS but more widely (Edmondson et al., 1994). The lack of myogenic conversion in this case is probably due to the presence of muscle-specific and brain-specific exons for this gene. To date only a single *myf-5* gene product is thought to be present in cultured cells and in embryos.

In differentiating ES cells, cultured in vitro, myf-5 transcripts have been detected at early stages preceeding by several days the appearance of skeletal muscle markers, such as MyoD, myogenin or myosin in embryoid bodies (Miller-Hance et al., 1993). Based on our observations, we would suggest that this early expression of *myf-5* is in neuronal cells that appear before skeletal muscle fibers in these cultures.

It is interesting that expression of *CeMyoD*, the only gene of this type identified in the nematode, occurs transiently in precursors of both neuronal and non-mesodermal cell types during *Caenorhabditis elegans* development. Subsequently, all descendants give rise to striated muscle, with the exception of six 'glial-like' cells that express *CeMyoD*, forming gap junctions with striated muscles in the head (Krause et al., 1992). Therefore, at least in this case, the fate of the *CeMyoD*-expressing cells is also not exclusively striated muscle.

Despite the different embryological origins of mammalian brain and skeletal muscle, several observations suggest that

these lineages may not be so distinct. A number of markers of early skeletal muscle are also expressed in neurons. Nestin, for example, is present in early neurons and in skeletal muscle cells, at the time when *myf-5* is also expressed in the myotome (Zimmerman et al., 1994). N-cam (Lyons et al., 1992), N-cadherins (Walsh et al., 1990), dystrophin (Houzelstein et al., 1992), and transcriptional factors such as engrailed (Logan et al., 1993) provide other examples. These observations suggest a certain degree of regulatory overlap between neuroectodermal and mesodermally derived tissues. Evidence provided by cultured cell lines further substantiate this idea. For example, the glial B9 (Lennon et al., 1979), cerebellar ST15A (Valtz et al., 1991) and embryonal carcinoma P19 (McBurney et al., 1982) cell lines yield neurons, glia and muscle in vitro, and the BC3H1 muscle cell line was derived from a brain tumor (Schubert et al., 1974). Some studies also suggest that differentiated muscle cells may be derived directly from primary brain or neural tube cultures (e.g. Ohanian, 1968; see Tajbakhsh et al., 1994). This flexibility between the two different programs is strikingly illustrated by our observations that *myf-5*-expressing cells in the neural tube, when cultured in vitro, will activate the myogenic program, initially expressing both neuronal and skeletal muscle markers in the same cell (Tajbakhsh et al., 1994). This phenomenon was not observed under similar culture conditions when brain cells expressing *myf-5* were grown in vitro (data not shown). This may be due to the culture conditions, but may also point to tighter maintenance of the in vivo restriction on myogenic conversion with these cells that represent distinct subdomains of brain tissue. In contrast, *myf-5*-expressing cells in the neural tube are more dispersed and are therefore more likely to differentiate in vitro in the absence of a community effect (Cossu et al., 1995).

Numerous transcription factors including members of the homeobox (Krumlauf, 1994) and *Pou* (Wegner et al., 1993) domain families are expressed in the developing brain, and have been implicated in segmentation (Fraser, 1993). Other genes identified to date do not display the same expression domains as *myf-5*, therefore this expression defines a distinct pattern. As in many of the other cases, it is difficult to correlate expression in the embryo with a known brain function. Strikingly, *myf-5* expression in the mammillary area is restricted to prosomere p4 providing further evidence that segmentation of the forebrain may be defined by gene-specific expression domains (Figdor and Stern, 1993; Puelles and Rubenstein, 1993). Members of the bHLH superfamily of genes, notably the achaete-scute complex in *Drosophila*, play an important role in neuronal cell determination (Ghysen and Dambly-Chaudiere, 1989). The mammalian homologue *MASH1* is expressed throughout the CNS although its role in neuronal cell specification is not clear (Guillemot et al., 1993). myf-5 may interact with this or other bHLH proteins to play a role in neurogenesis.

It is noteworthy that the onset of *myf-5* expression in the brain is at the open neural head fold stage, coincident in time and location with the appearance of the first neuronal cells (Easter et al., 1993). The ontogeny of the regions in the brain where *myf-5* is expressed is poorly understood, mainly because there were no cell lineage markers. The restricted expression of *myf-5* to the posterior hypothalamus suggests that β-gal$^+$ cells in the mammillary area of prosomere p4 will later localise to this specific region in the older brain. Whether continued *myf-5* expression in the hypothalamus is critical for the animal awaits detailed analysis of homozygous mutant mice. Our preliminary analysis of homozygous embryos indicates no major perturbations in the developing brain. The myf-5$^-$/β-gal$^+$ expressing cell population is present. *myf-5* homozygous mutant mice die at birth probably due to rib defects arising as a secondary consequence of the impairment of early myogenesis (Braun et al., 1992; S.T., D. Rocancourt, and M.B. unpublished observations). It may prove possible in the future to specifically target brain expression of *myf-5*, perhaps via a potential neuronal-specific regulatory element (c/f nestin (Zimmerman et al., 1994), engrailed (Logan et al., 1993) or dystrophin (Barnea et al., 1990)), to look specifically at potential effects on brain function in older mutant animals.

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