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Review

Pax genes in myogenesis: Alternate transcripts add complexity

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Summary. Pax3 and Pax7 are powerful myogenic inducers and hence play a critical role in skeletal muscle development and regeneration. In this paper we discuss the role of Pax3 and Pax7 in dorsal patterning of the somite with subsequent determination of myogenic precursor cells for muscle formation within the developing embryo and in adult muscle. Recent evidence of the ability of stem cells to contribute to muscle regeneration in adult tissues, and the role of Pax7 in conversion of multipotent stem cells to the myogenic lineage are also discussed.

Several tissue specific *Pax7* transcripts that encode isoforms with different DNA binding characteristics and potentially distinct transactivation specificities are identified. The expression of a range of transcripts in the determination of different tissue lineages and distinct cell populations both in the embryo and in the adult indicates an extraordinary level of complexity. A detailed understanding of these molecules and their functions during embryogenesis and adult muscle formation is imperative for future stem cell therapies.

Key words: Myogenesis, Pax7, Pax3, Satellite cell, Alternate transcripts

Introduction

Developmental *Pax* genes encode highly conserved transcription factors that regulate early embryonic pattering, a function first described for the homologous *Drosophila melanogaster*, *prd* gene (Bopp et al., 1986). *Pax* genes also play a role in organ and tissue specification, regulating cellular proliferation and differentiation, ultimately specifying subsets of cells within discrete tissue locations. *Pax* genes are grouped into four classes based on the structure of their functional domains (Fig. 1) and members within each class may exhibit partial functional redundancy.

This review explores the ability of Group III genes, *Pax7*, and to a lesser extent, *Pax3*, to specify myogenic precursor cells during vertebrate skeletal muscle development, growth and regeneration. The inductive properties of Pax7 may be utilized in future stem cell therapies for the treatment of an array of muscle diseases and injuries.

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Pax3 and *Pax7* in embryonic myogenesis: Specification of distinct muscle precursors

Skeletal muscles are formed from the paraxial mesoderm that surrounds the neural tube. The caudal mesoderm condenses to form somites that differentiate dorsally into epithelial dermomyotome and ventrally into mesenchymal sclerotome (Tremblay et al., 1998). Cells of the dermomyotome exhibit early, restricted expression patterns of *Pax3* and *Pax7* and develop into dermis or skeletal muscle of the trunk and limb (Fig. 2; Ordahl and LeDouarin, 1992; Parker et al., 2003). By contrast, *Pax1* expressing cells of the sclerotome develop into cartilage or bone of vertebra and rib (Deutsch et al., 1988).

Clear evidence exists for the role of *Pax* genes in patterning of the neural tube and brain from which specific neurons of the central nervous system are derived (Jostes et al., 1990; Jessel and Sanes, 2000; Thomas et al., 2004), and it appears that early restricted expression patterns of *Pax3* and *Pax7* similarly pattern the somites from which distinct skeletal muscle progenitors are formed (Jostes et al., 1990; Kawakami et al., 1997). Initially, *Pax3* and *Pax7* are expressed ubiquitously throughout the dermomyotome (Goulding et al., 1994); later *Pax3* becomes down-regulated dorso-medially and is predominantly expressed in the lateral dermomyotome, whereas *Pax7* expression becomes concentrated in the dorso-medial region of the dermomyotome (Kaehn et al., 1988; Jostes et al., 1990; Goulding et al., 1994; Tajbakhsh et al., 1997; Tremblay et al., 1998; Chi and Epstein, 2002).

Cells of the dermomyotome give rise to non-

migratory and migratory progenitors for the development of skeletal muscle. Non-migratory progenitors are produced by proliferation of dermomyotomal cells at the dorso-medial lip (DML) and ventro-lateral lip (VLL) and extension of these cells beneath the dermomyotome form the epaxial and hypaxial myotome respectively. While *Pax7* positive cells of the epaxial dermomyotome terminally differentiate to form dorsal trunk muscle, *Pax3* positive cells of the hypaxial dermomyotome establish lateral trunk muscle. Thus body wall musculature is derived from non-migratory *Pax3* and *Pax7* expressing cells of the myotome (Fig. 2, Selleck and Stern, 1991; Goulding et al., 1994; Denetclaw et al., 1997; Tremblay et al., 1998; Houlzestein et al., 1999; Denetclaw and Ordahl, 2000; Parker et al., 2003).

Migratory, muscle progenitor cells arise from VLL cells that undergo an epithelial to mesenchymal transition, delaminate and migrate to form muscles of the ventral body wall (hypaxial), or diaphragm, tongue and limbs. Hypaxial cells migrate en masse to the ventral body wall, while long-range somitic cells migrate in waves to their final destination, where terminal differentiation occurs after the expression of muscle determination genes, *Myf5*, *MyoD*, *MRF4* and *Myogenin* (Christ and Ordahl, 1995; Ordahl et al., 2000; Bailey et al., 2001). *Pax3* expression is crucial for specification of both hypaxial and long-range migrating cell types (Goulding et al., 1994; Tremblay et al., 1998; Cinnamon et al., 1999).

Pax7 positive, migratory cells are apparent at later stages of limb development, and are thought to be associated with the formation of limb muscles, or with specification of impending satellite cells, dormant muscle precursors found in mature muscle, and associated with postnatal muscle growth and

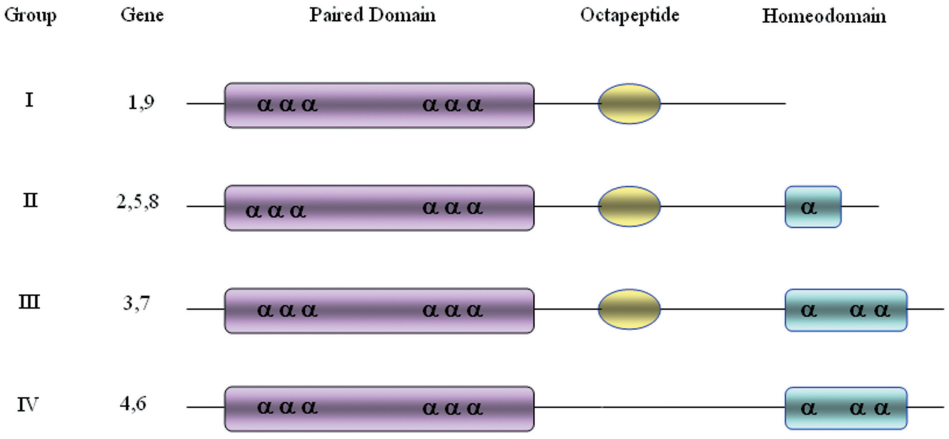

Fig. 1. Protein domains encoded by Pax groups I – IV. The paired domain is characteristic of all Pax proteins. The homeodomain occurs as a truncated form, with one alpha helix (Pax group II) or as a full homeodomain, containing three alpha helices (Pax groups III and IV). The octapeptide is found in Pax groups I, II and III (adapted from Mansouri et al., 1996).

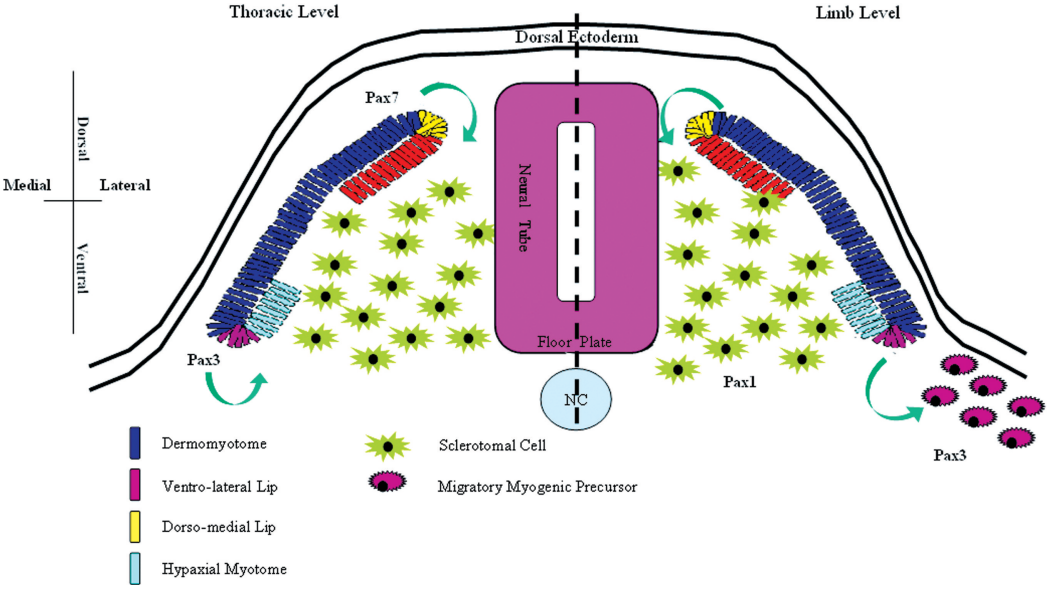

Fig. 2. A diagram of *Pax* gene expression during muscle development in the embryonic somite: *Pax7* expressing cells of the dorsal medial dermomyotome produce epaxial myotome and hence dorsal body wall musculature. *Pax3* expressing cells of the ventral lateral dermomyotome give rise to hypaxial myotome and hence muscles of the ventral body wall. In somites at the limb level, *Pax3* expressing cells of the ventral lateral lip migrate to form muscles of the developing limb, diaphragm and tongue. *Pax1* is expressed in sclerotomal cells, which develop into cartilage and bone of the vertebrae and ribs (Diagram adapted from Chi and Epstein, 2002).

regeneration (Ordahl and LeDouarin, 1992; Christ and Ordahl, 1995; Tsonis, 2002).

The crucial roles that *Pax3* and *Pax7* play in myogenesis are highlighted by the mutated phenotype. In *Splotch* (*Pax3*$^{-/-}$) mice, limb and ventral trunk muscles are completely absent. However, lateral trunk muscles, derived from non-migratory cells of the hypaxial myotome (*Pax3* expressing in the wild-type), develop normally (Tremblay et al., 1998). Similarly, dorsal body wall muscles, typically specified by *Pax7*$^{+}$, non-migratory cells of the epaxial myotome, develop normally in the absence of *Pax7*, yet satellite cells, derived from migrating cell populations are completely absent in *Pax7* null C57/Bl mice (Seale et al., 2000; Parker et al., 2003). Therefore it is apparent that either paralogous gene is able to induce myogenic differentiation in non-migratory cells for the formation of trunk muscles. However, compensation is not possible during activation of pathways required for specification of migrating cell populations, implying that activation of explicit downstream factors involved in cell migration and specification are crucial for completion of appendicular myogenesis.

Secretory signals pattern somite development and regulate *Pax* gene expression

Proteins secreted from signalling centres in the embryonic neural tube, notochord, ectoderm and mesoderm mediate dorso-ventral and medio-lateral patterning to bring about dermomyotome formation, hypaxial and epaxial myotome determination, and specification of lateral dermomyotomal cells that become migratory appendicular cells. This is achieved by regulating expression of genes, including *Pax* genes, to control temporal and spatial proliferation and differentiation of muscle progenitor cells; extensive proliferation is required to produce appropriate quantities of anatomical muscle in coordination with bone and tendon formation. Moreover, myogenesis within migratory precursor cells needs to be delayed until the cells have arrived at their sites of muscle differentiation.

While there is much controversy over the exact role of signalling proteins in myogenesis, it is generally agreed that BMPs inhibit, while Shh and Wnts activate skeletal muscle differentiation in the somites (Tajbakhsh, 2003). BMPs, specifically BMP-2, BMP-4 and BMP-7, secreted from the lateral plate mesoderm (Cossu et al., 1996), inhibit myogenic differentiation and *MyoD* expression (Pourquie et al., 1996) ensuring continued proliferation and migration of undifferentiated cells. Although the role of BMPs in myogenesis is not altogether clear, there is evidence that BMP-4 activates *Pax7* in a concentration dependent manner (Timmer et al. 2002). The continued expression of *Pax7* (or *Pax3*) within progenitor cells would ensure their continued proliferation, as opposed to their differentiation. An association between Pax3/Pax7 and cell proliferation has been confirmed in myoblast cells in culture, as well as in *MyoD*$^{-/-}$ mice, in which satellite cells proliferate but do not differentiate and *Pax7* expression increases four-fold (Epstein et al., 1995; Seale et al., 2000).

By contrast, inductive signals Wnt and Shh mediate myogenic differentiation; either molecule is sufficient to induce myogenesis in cultured somites (Munsterberg et al., 1995). Wnt peptides (Wnt1 or Wnt3) are released from the neural tube and Shh is released from the floor plate/notochord (Tajbakhsh, 2003). Shh induces terminal differentiation specifically in epaxial non-migratory progenitor cells by early up-regulation of *Myf5* and *MyoD* (Chiang et al., 1996; Borycki et al., 1999). Shh is thought to activate the expression of *Myf5* essential for *MyoD* activation via Gli1; Gli1 binding sites have been identified in the enhancer region of the *Myf5* gene (Gustafsson et al., 2002). Proteins secreted from the dorsal ectoderm also stimulate terminal myogenic differentiation in axial precursor cells. Noggin, a BMP antagonist secreted from the dorsal medial lip, under the control of Wnt signalling from the surface ectoderm, activates the expression of *MyoD* with subsequent down-regulation of *Pax3* in non-migratory hypaxial cells, and up-regulates *Myf5* expression in non-migratory epaxial cells (Cossu et al., 1996).

The required factors and progressive steps for myogenic determination have been delineated by functional studies in P19 carcinoma cells; Wnt3 up-regulates *Pax3* which in turn activates *Six1*, *Eya2* and *Dach2*, followed by down-regulation of *Pax3* and activation of the expression of *MyoD* and *myogenin* (reviewed in Parker et al., 2003). Several experiments confirm these findings; for example, Wnt up-regulates *Pax3* and *Pax7* expression in presegmented, paraxial mesoderm explants and ectopic expression of *Pax3* in paraxial and lateral plate mesoderm induces expression of *MyoD*, *Myf5* and *myogenin*, without Shh or Wnt signals (Maroto et al., 1997).

The steps along a myogenic pathway are different for migrating cells. These dermomyotomal cells adopt a mesenchymal morphology, delaminate and migrate to the limb bud, while retaining their myogenic specification with protracted *Pax3* or *Pax7* expression until their target sites are reached. Once within the limb bud, cellular differentiation progresses in a proximal to distal direction as the limb develops and elongates. The proximal to distal wave of differentiation correlates with expression of signalling proteins of the FGF family, including FGF2, 4 and 8, from the apical ectodermal ridge. Although several experiments indicate that FGF inhibits myogenic determination, recent experiments show that it is essential for terminal differentiation in the limb bud, being required for transition from a *Pax3*+ uncommitted state to a *MyoD*$^{+}$ committed state (Epstein et al., 1995; Francis-West et al., 2003).

Clearly, controlled expression of BMPs, Wnts, FGFs, Shh and noggin act in concert with *Pax* genes, to bring about the differentiation of the somite across the dorso-ventral and medio-lateral axes and ensures the

appropriate expansion and differentiation of distinct myogenic cells. However, the ability of Pax3 and Pax7 to direct the dorso-ventral differentiation of both the neural tube and somite would indicate the necessity for cooperation with region specific cues and localised co-factors for appropriate differentiation of area-specific cell types. In the anterior region of the neural tube, for example, Pax3 and Pax7 act together with Otx2, En, Fgf8, Pax2 and Ephrin-A2 to differentiate the midbrain from the forebrain and hindbrain (Matsunaga et al., 2001; Thomas et al., 2004). Similarly, the somite requires expression of myogenic determination genes such as *Pax3* and *Pax7* together with *Six1*, *Eya1*, *Dach2*, *Msx1*, *Myf5* and *MyoD* or the negative regulator *myostatin* (Bmp-8), for specification of muscle precursor cells (Heanue et al., 1999; Houzelstein et al., 1999; Thomas et al., 2000; Kardon et al., 2002; Parker et al., 2003).

Migrating cell populations and migratory signals

Three distinct migratory myogenic progenitor cells have been identified - embryonic and fetal myoblasts and satellite cells and together these cells contribute to the formation and maintenance of ventral trunk, limb, diaphragm and tongue skeletal muscle. Embryonic and fetal myoblasts contribute to myogenesis in the embryo, with embryonic myoblasts forming primary myofibres, and fetal myoblasts giving rise to secondary myofibres (Parker et al., 2003; Smith et al., 1993). However, these cells are disparate in terms of gene expression, morphology, drug resistance and origin (Goulding et al., 1991; De Angelis et al., 1999; Hawke and Garry, 2001; Buckingham et al., 2003); migratory embryonic myoblasts arise from *Pax3* positive dermomyotomal cells by epithelial to mesodermal transition whereas it has been suggested that fetal myoblasts are multipotential cells of endothelial origin (De Angelis et al., 1999).

Satellite cells, first identified by Mauro (1961) and specified by Pax7 rather than Pax3, persist after the completion of myogenesis, residing unsheathed as dormant myonuclei beneath the basal lamina of myofibres (Schultz and McCormick, 1994; Hawke and Garry, 2001). *Pax7* expression, observed in migrating cells, continues in quiescent and activated satellite cells, only becoming down-regulated upon terminal differentiation of the cells. Satellite cells may arise from a small population of *Pax7* positive epaxial migratory cells, or from migratory VLL cells that originally express *Pax3*, or alternately, from cells of an endothelial origin; endothelial cells of the embryonic dorsal aorta possess a similar morphology and gene expression profile to satellite cells (De Angelis et al., 1999). Further studies including fate-mapping experiments are required to determine their origin.

Secretory factors, fibroblast growth factor (FGF) and hepatocyte growth factor (HGF), expressed from the lateral plate mesoderm and limb bud, provide chemotactic cues for delamination and migration of appendicular cells (Brand-Saberi et al., 1996; Heymann et al., 1996). Migrating cells express the HGF receptor *c-met*, crucial for delamination, as well as *Lbx1* important for migration, both genes being activated, directly or indirectly, by Pax3 (Epstein et al., 1996). *c-Met* is also co-expressed with *Pax7* in quiescent satellite cells, consistent with the proposed role for the ligand (HGF) in satellite cell activation and migration (Cornelison and Wold, 1997; Seale and Rudnicki, 2000). Other cell surface molecules important in the migratory process include Eph receptors and ephrins (Swartz et al., 2001) and recent experiments indicate their up-regulation by Pax7 in the developing embryo (Thomas et al., 2004) and their co-expression with *Pax7* in satellite cells of adult muscle (Ziman, personal observation).

Pax7 positive satellite cells in postnatal myogenesis and regeneration

The remarkable adaptive ability of postnatal skeletal

Table 1. Role of immune cells, cytokines and growth factors in the various stages of satellite cell ontogeny for muscle growth and repair.

SIGNAL	STAGE IN SATELLITE CELL ONTOGENY	REFERENCES
Immune Cells		
Macrophages, Lymphocytes, Leukocytes	Activation	Orimo et al., 1991; Robertson et al., 1993; Tidball, 1995; Jesse et al., 1998; Merly et al., 1999; Skuk and Tremblay, 2000
Growth Factors and Cytokines (For review, see Husmann et al., 1996)		
HGF	Activation, proliferation, migration	Cornelison and Wold, 1997; Tatsumi et al., 1998; Seale and Rudnicki, 2000; Marics et al., 2002
FGF	Activation, proliferation	Allen and Boxhorn, 1989; Groux-Muscatelli et al., 1990; Robson and Hughes, 1996; Cornelison and Wold, 1997; Floss et al., 1997; Marics et al., 2002
IGF-1, IGF-2	Proliferation, differentiation	Jennische et al., 1987; Florini et al., 1991; see Florini et al, 1996 for review; Grounds 1999; Musaro and Rosenthal, 1999; Musaro et al., 2001; Chakravarthy et al., 2000
IL-6, LIF	Proliferation	Cantini et al., 1994; Kurek et al., 1996, 1998; Austin et al., 2000

muscle to physiological demands such as normal growth, growth after intensive exercise, and regeneration after injury, is attributable to the efficacy of satellite cell activation, proliferation, differentiation and fusion (Cossu et al., 1988; Schultz and McCormick, 1994; Cornelison and Wold, 1997; Seale et al., 2000; Hawke and Garry, 2001). In response to myotrauma, due to a muscle tear or myopathy for example, signals from immune cells and the extracellular matrix stimulate conversion of the satellite cell from a quiescent to an activated state (Table 1; Fig. 3). This induces an asymmetrical division that results in replacement of a quiescent satellite cell and production of an activated muscle precursor. The resulting muscle precursor cell migrates to the site of injury, differentiates, and fuses to existing muscle fibres, thus effecting repair (Cornelison and Wold, 1997; Grounds, 1999; Seale and Rudnicki, 2000; Hawke and Garry, 2001). It is believed that satellite cells have a finite capacity for self-renewal (Buckingham et al., 2003); in pathological cases where the demand for fibre repair is ever-present such as in Duchenne Muscular Dystrophy, the satellite cell pool becomes exhausted and the muscle eventually fails to regenerate resulting in loss of muscle function and death (Hartigan-O'Connor and Chamberlain, 2000).

While the role of *Pax7* in embryonic muscle formation is not completely understood, *Pax7* is strongly implicated in postnatal muscle growth and repair, being expressed in satellite cells during their specification, proliferation and activation (Conboy and Rando, 2002; Tajbakhsh, 2003). Skeletal muscles of *Pax7-/-* C57/BL mice develop with relatively normal organisation, but postnatal muscle is completely lacking in satellite cells and proliferating myoblasts. Such *Pax7-/-* mice are significantly smaller than the wild-type (50 percent at seven days old), the diaphragm develops to half the normal thickness and limb muscle fibres show a marked decrease in muscle mass. These adverse effects strongly support the hypothesis that *Pax3* compensates for loss of *Pax7* in axial muscle determination but not in satellite cell mediated postnatal skeletal muscle growth and regeneration, this being severely restricted in the absence of *Pax7* (Seale et al., 2000).

The transition of satellite cell from the quiescent to the activated state occurs with the requirement for muscle regeneration and the sequence of events is orchestrated by many factors including growth factors, and myogenic regulatory factors (Table 1). For example, myogenic regulatory factors (Myf5, MyoD, myogenin and MRF4) are only found in activated satellite cells where they mediate the up-regulation of skeletal muscle specific genes and induce differentiation of satellite cells (Cornelison and Wold, 1997; Sabourin and Rudnicki, 2000; Seale and Rudnicki, 2000). Using microarray analysis, the factors and genes expressed during regeneration of skeletal muscle have been characterised. From this data it is evident that regeneration does not recapitulate development; no positional cues (*Wnt*, *Shh* or *BMP*s) are expressed, whereas *Pax7* and *FGFR-4*, cell autonomous factors that regulate cell proliferation and myogenic determination, are highly expressed during regeneration (Zhao and Hoffman, 2004).

It is debatable whether satellite cells are able to provide all of the myoblasts required during skeletal

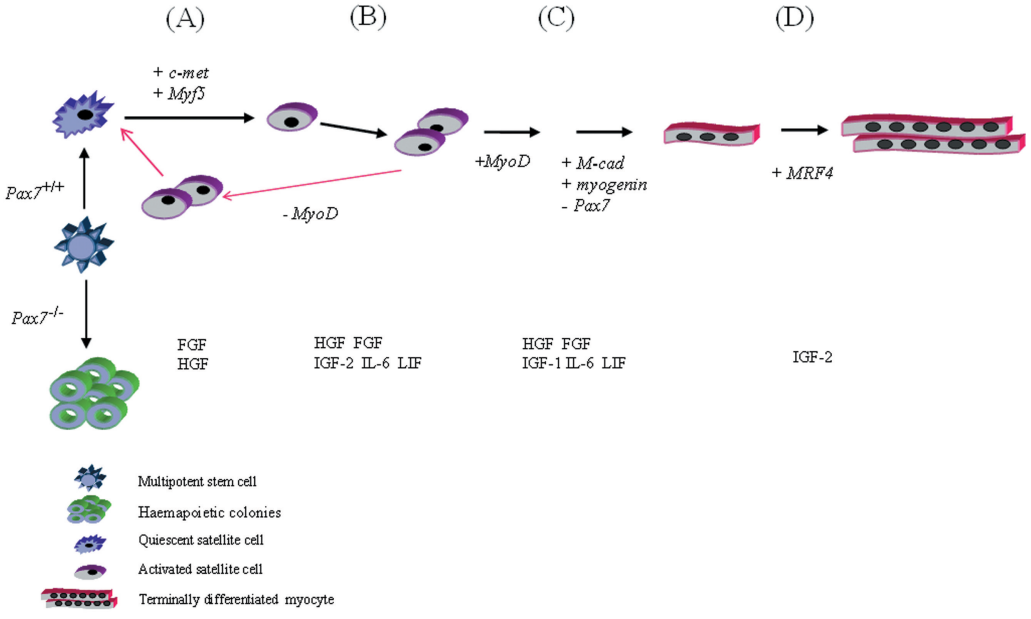

Fig. 3. A diagrammatical representation of factors that modulate satellite cell ontogeny. Satellite cells are specified from pluripotent stem cells by the expression of *Pax7*. Satellite cells become activated **(A)** by growth factors (upper case), with concomitant up-regulation of *MRF*'s. The cells proliferate **(B)** under the guidance of cues including IGF-2, IL-6 and LIF. Upon the expression of *MyoD* and subsequently *myogenin* and *M-cad*, cells begin to differentiate, and **(C)** *Pax7* becomes down-regulated. Terminal differentiation **(D)** is stimulated by signals from IGF-2 and up-regulation of *MRF4*. The HGF receptor, *c-Met*, implicated in stimulating cell migration, is expressed in satellite cells, throughout the entire process of ontogeny. Alternatively, activated satellite cells in which *MyoD* is not up-regulated, revert to their quiescent state after proliferation concomitant with asymmetric division for satellite cell renewal (open arrowheads).

muscle repair; in fact recent experiments have shown that other non-satellite stem cells resident in the muscle, may replenish the satellite cell compartment and thus participate in muscle regeneration. Whereas muscle satellite cells are the dominant source of myogenic precursors, under extreme circumstances, muscle side-population (SP) or bone marrow stem cells may also be incorporated into regenerating skeletal muscle and their incorporation is characterised by up-regulation of *Pax7* (Ferrari et al., 1998; Asakura et al., 2002; LaBarge and Blau, 2002; Polesskaya et al., 2003; Musaro et al., 2004).

Pax7 structure and functional domains

The transcription factors Pax3 and Pax7 activate myogenesis by binding to the promoter region of target genes, and hence facilitate the formation of a DNA-protein pre-initiation complex that activates transcription of the target gene. Pax3 and Pax7 are classified as Group III Pax proteins as they contain a paired and a homeodomain, a conserved octapeptide and a transactivation domain encoded by eight or nine exons (Balczarek et al., 1997). Binding to target DNA is through the N-terminal paired and homeodomains (Schafer et al., 1994; Xu et al., 1995; Jun and Desplan, 1996; Chi and Epstein, 2002); the two sub-domains within the paired domain each contain a helix-turn-helix (HTH) motif, and the homeodomain contains a single HTH motif (Fig. 4a,b). Group III Pax proteins utilise either the paired subdomains or the homeodomain or a combination of these to bind different target DNA sequences (Treisman et al., 1991; Schafer et al., 1994; Xu et al., 1999; Ziman et al., 2001b). Research demonstrates that the binding specificity of the paired domain may influence the binding specificity of the homeodomain (Underhill and Gros, 1997; Mishra et al., 2002), known to facilitate cooperative dimerisation on palindromic sites (Gehring, 1993; Jun and Desplan, 1996; Chi and Epstein, 2002).

Protein-protein interactions of the Pax transactivation domain, with a variety of transcription factors activate transcription of the target gene (Schafer et al., 1994; Cho et al., 2003). The transactivation domain is a Proline, Threonine, Serine rich region at the C-terminus of the Pax protein (Jostes et al., 1990; Seo et al., 1998), and it is estimated that these amino acids are target sites for phosphorylation and other post-translational modifications that mediate the function of this domain (Mikkola et al., 1999).

Recent experiments have identified several Pax3 and Pax7 isoforms with alternate N- and/or C-termini, produced by alternate splicing in the gene regions encoding the paired and transactivation domains (Vogan et al., 1996; Vogan and Gros, 1997; Vorobyov et al., 1997; Ziman et al., 1997; Barber et al., 1999; Barr et al., 1999). The alternate isoforms differ in structure as well as in their ability to bind and activate target genes (Schafer et al., 1994; Vogan et al., 1996; Vogan and Gros, 1997; Ziman and Kay, 1998; Barber et al., 1999; Barr et al., 1999; Ziman et al., 2001a; Chi and Epstein 2002). The presence of conserved alternate transcripts with similar function throughout phylogeny highlights their significance.

Alternate *Pax7* paired box transcripts

Recent research has identified several alternate transcripts for *Pax7* and these will be discussed here in detail.

Four *Pax7* transcripts produced by alternate splicing of the paired box, *Pax7a-d*, have been characterised in embryonic mice and adult mice and humans. Alternate splicing of *Pax7* pre-mRNA within the paired box produces transcripts that include or exclude a tri-nucleotide, CAG, or a hexa-nucleotide, GTTTAG, at the beginning of the second and third exon respectively (Vogan et al., 1996; Vogan and Gros, 1997; Ziman and Kay, 1998; Ziman et al., 2001a). The encoded isoforms

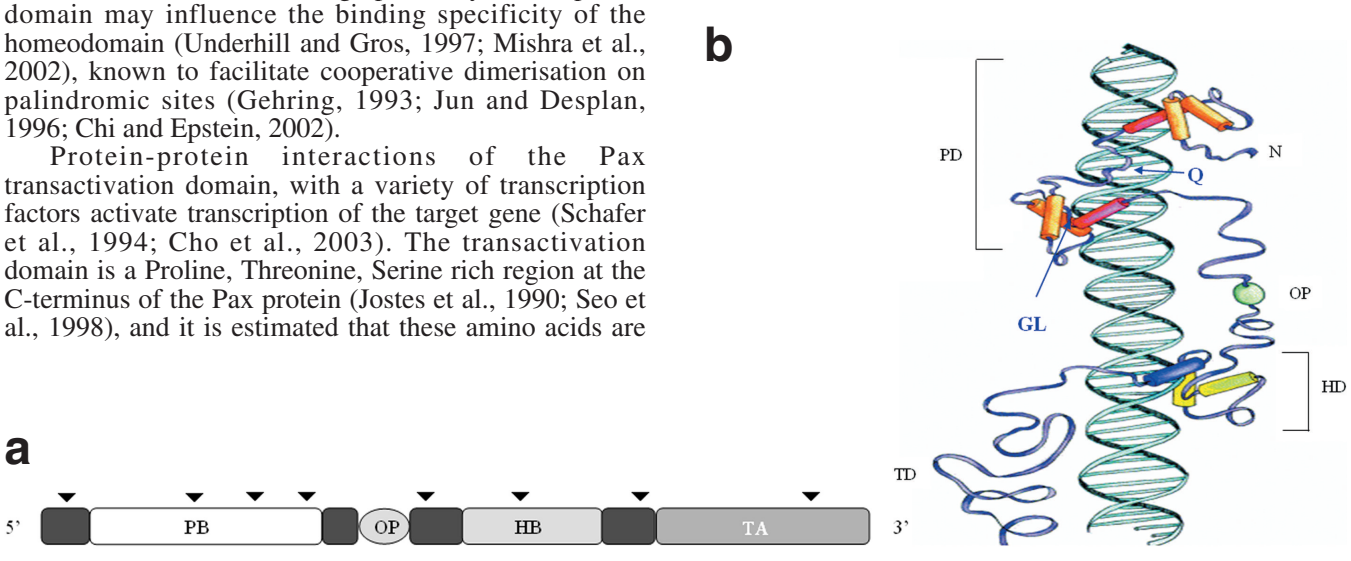

Fig. 4. *Pax7* genomic and protein structure. **a.** Genomic structure of *Pax7*, 5' to 3': The *Pax7* gene consists of a paired box (PB) encoding the paired domain, a conserved octapeptide encoding region (OP), a homeobox (HB) encoding the homeodomain, and the region encoding the C-terminal transactivation domain (TA). Intron exon boundaries for exons 1-9 are indicated by arrowheads (6); **b.** The encoded transcription factor functional domains include the DNA binding, paired and homeodomains (PD and HD respectively), the octapeptide (OP) and the transactivation domain (TD) (adapted from Chi and Epstein, 2002).

include or exclude a glutamine (Q) and/or a glycine-leucine (GL) dipeptide within the paired DNA binding domain (Figs. 4b, 5). The addition of glutamine (Q) increases the length of the linker region between the two HTH motifs of the paired domain and alters the binding affinity of the second HTH motif. The inclusion of the dipeptide (GL) increases the length of the third alpha helix in the second HTH motif and may alter the binding of the Pax7 paired domain to target genes (Vogan et al., 1996; Ziman and Kay, 1998). The various isoforms of Pax7 possess distinct tertiary structures, have differential DNA binding affinities and specificities and therefore, presumably activate different genes (Noll, 1993; Vogan et al., 1996; Ziman and Kay, 1998; Barr et al., 1999; Ziman et al., 2001a).

Pax7 isoforms with alternate transactivation domains

Alternate splicing of the region encoding the transactivation domain has been reported for many *Pax* genes and the encoded isoforms, containing alternate C-termini, are thought to exhibit diverse transactivation properties (Poleev et al., 1995; Barr et al., 1999; Pritchard et al., 2003); in particular, Pax3 (Barber et al., 1999; Pritchard et al., 2003) as well as Pax8 (Kozmik et al., 1993) isoforms demonstrate differential transcriptional activity.

Transcripts of *Pax7* with alternate 3' ends have been identified in human, mouse and zebrafish (Vorobyov et al., 1997; Seo et al., 1998; Barr et al., 1999; Seale et al., 2000). In humans, one transcript *PAX7A*, contains exons one through to eight, whilst the other, *PAX7B* contains exons one through to nine, where the eighth exon is spliced at a conserved site and joined to an additional ninth exon (Barr et al., 1999). These alternative products encode proteins that possess distinct C-termini (Fig. 6).

Murine *Pax7A* and *Pax7B* transcripts also contain eight (NCBI: AF453394) and nine exons (Seale et al., 2000) respectively. Murine Pax7B is analogous to human PAX7B, showing 97% homology overall and 100% homology at the C-terminus. By contrast, murine Pax7A contains a unique C-terminus with only 7% homology to the human isoform in the region downstream of the eighth exon (Fig. 6), and 96% homology in the regions encoded by exons up to this point (Barr et al., 1999). The functional significance of the alternate C-termini remains to be determined; the high level of conservation in the DNA binding domains implies functional conservation, while the alternate C-termini indicate differences in transactivation activity.

Tissue specificity of *Pax7* transcripts

Varied expression levels of the alternate *Pax7* transcripts that are observed in both embryonic and adult muscle, would imply differences in function. While all transcripts, both paired and 3'-variants, are expressed in embryonic and adult limb and diaphragm muscle of mice (Ziman and Kay, 1998; Ziman et al., 2001a; Lamey et al., 2003), the relative expression levels of the paired transcripts vary considerably; for example *Pax7c* is consistently expressed at lower levels than *Pax7b* and *Pax7d*. Moreover individual *Pax7* transcript levels are significantly different within brain and skeletal muscle of mice, implicating them in the regulation of cell differentiation along these lineages.

Using an *in vitro* model system we showed that different *Pax7* transcripts are indeed required for differentiation along neurogenic and myogenic lineages; while specific transcripts are present in neurons, muscle cells and normal embryonic and adult muscle tissues consistently express all transcripts, including those with alternate 3' ends (Table 2, Ziman and Kay, 1998; Ziman 2001a; Lamey et al., 2003).

It is tempting to speculate that alternate *Pax7* transcripts have distinct roles at certain stages of ontogeny of the myogenic precursor, since all transcripts are present in normal muscle. However, at this stage it remains unclear whether all transcripts are equally important in determining myogenic differentiation, or if myogenic differentiation can proceed with expression of individual transcripts. To establish the importance of individual transcripts in the differentiation process, a single full length *Pax7* transcript, *PAX7b* (from human skeletal muscle) was transfected into P19 cells; however this induced the cells to differentiate along a neurogenic lineage (Ziman et al., 2001a). By contrast, recent *in vitro* experiments indicate that individual alternate transcripts are able to induce a myogenic lineage in embryonic cell

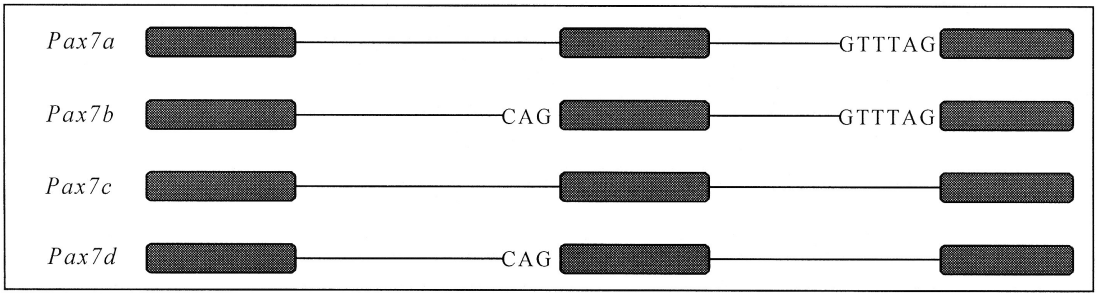

Fig. 5. Alternate transcripts of the *Pax7* paired box produced by differential splicing events that lead to the inclusion or exclusion of a tri-nucleotide (CAG) or a hexa-nucleotide (GTTTAG), (adapted from Ziman et al., 2001b).

lines, however these experiments remain to be confirmed (personal observation).

Conclusion

Pax Group III expression (both *Pax3* and *Pax7*) in differentiating precursor cells of developing, maturing and regenerating vertebrate skeletal muscle is relatively well established. However, the binding promiscuity of Pax proteins to target gene promoter sequences via the PD, or the HD or both, coupled with alternate isoform binding and transactivation efficiency means that activation of target genes is compounded by enormous complexity and diversity.

Whereas several studies have focused on the spatio-temporal expression patterns of *Pax3* and *Pax7* during development and in adult tissues (Jostes et al., 1990; Denetclaw et al., 1997; Kawakami et al., 1997; Mootoosamy and Dietrich, 2002) few have focussed on the spatio-temporal expression patterns of the individual *Pax7* transcripts, yet these may be fundamental in the effectiveness of muscle development and regeneration.

It is important to elucidate the role of individual *Pax7* transcripts in embryonic and postnatal myogenesis since this may have implications for the use of *Pax7* as a candidate gene for the conversion of stem cells to the myogenic lineage, a treatment strategy for myopathies aimed at significantly enhancing muscle regeneration.

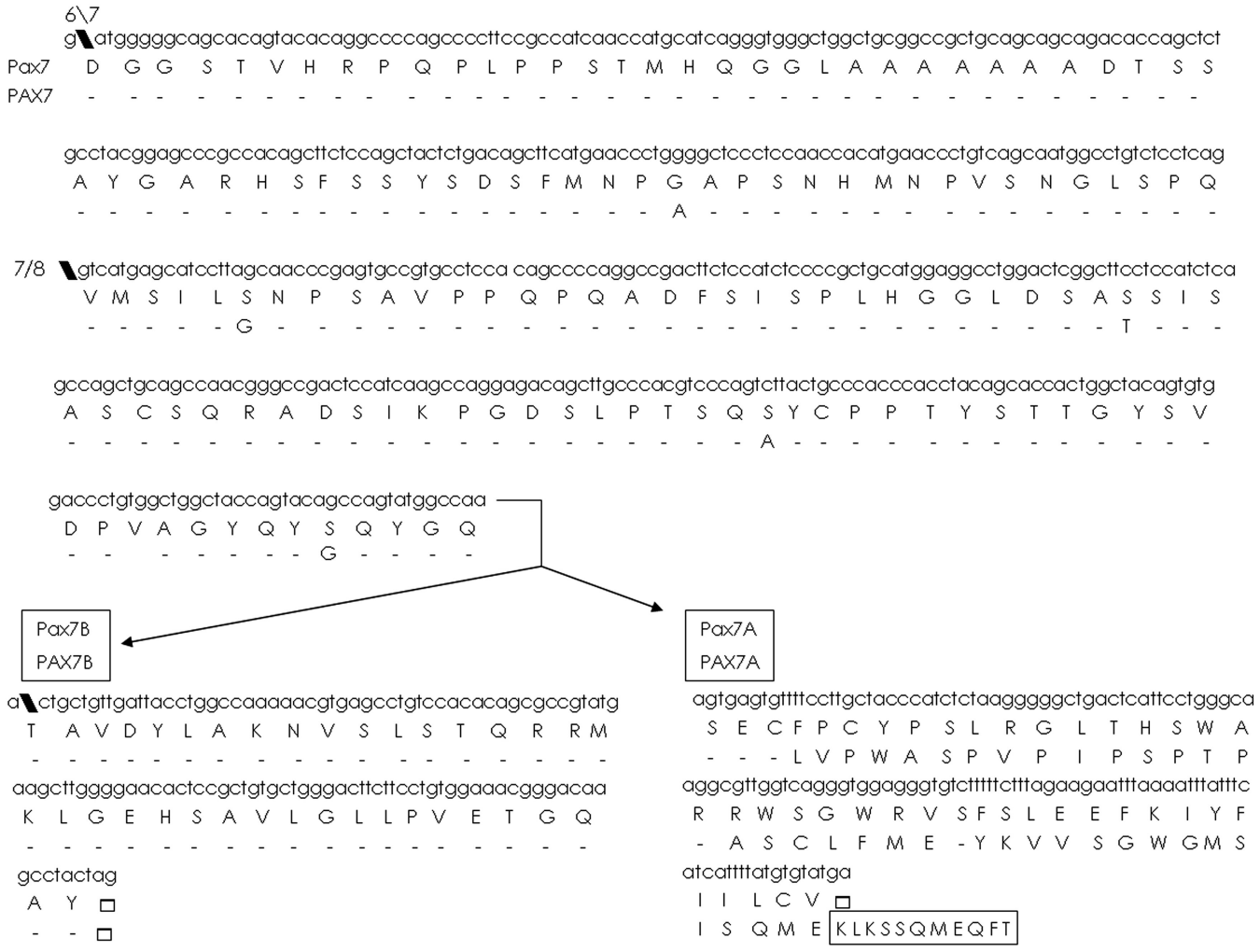

Fig. 6. Murine *Pax7* mRNA (NCBI: AF254422) downstream of the exon 6/7 boundary (\) with the predicted amino acid sequence indicated below. The human protein sequence is homologous, differing by only five amino acids from the exon 6/7 boundary to the 8/9 boundary (8/9 \), as indicated by the appropriate amino acids. The alternate murine transcripts, *Pax7A*, *Pax7B*, diverge midway through exon eight at the exon 8/9 boundary. Whilst the B isoforms of human and mouse, encoded by nine exons, are highly conserved, the A isoforms, encoded by eight exons, diverge midway through exon eight shortly after the alternate splice site. Human PAX7A encodes an additional 11 amino acids (boxed) relative to the murine Pax7A isoform before translation is terminated at a stop codon (❐).

Table 2. Relative expression levels (%) of alternate paired box *Pax7* transcripts (a-d) in mouse and human skeletal muscle as identified by RNA isolation and subsequent RTPCR, cloning and sequencing. It is interesting to note that the transcript profile differs markedly in skeletal muscle of wild-type mice, possibly indicating a difference in expression of transcripts between normal (quiescent, BALB/c) and regenerating muscle (SJL/J). *Pax7c* is consistently expressed at lower levels in all models. Studies *in vitro* show a similar trend; P19 cells differentiated along a myogenic lineage (by treatment with DMSO) express all four transcripts with *Pax7c* expressed at the lowest level and *Pax7b* at the highest level. Interestingly, P19 cells differentiated along a neurogenic lineage (by treatment with RA) are completely devoid of *Pax7a* and *Pax7c* expression, and Pax7d is expressed at higher levels than *Pax7b* (adapted from Ziman and Kay, 1998; Ziman et al., 2001a).

MODEL	*Pax7a*	*Pax7b*	*Pax7c*	*Pax7d*
Human	5.5	28	5.5	61
Mouse (BALB/c)	25	29	14	32
Mouse (SJL/J)	5	85	0	10
DMSO treated P19 cells	12	49	9	30
RA treated P19 cells	0	44	0	56

The *ex-vivo* growth of skin for transplantation is already in wide clinical use. It follows that a similar approach may also be used for muscle tissue engineering.

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