Pax genes: Regulators of lineage specification and progenitor cell maintenance

Judith A. Blake  
*Edith Cowan University*

Mel R. Ziman  
*Edith Cowan University*, m.ziman@ecu.edu.au

Follow this and additional works at: [https://ro.ecu.edu.au/ecuworkspost2013](https://ro.ecu.edu.au/ecuworkspost2013)

Part of the [Medicine and Health Sciences Commons](https://ro.ecu.edu.au/ecuworkspost2013)

10.1242/dev.091785  
This Journal Article is posted at Research Online.  
PRIMER

Pax genes: regulators of lineage specification and progenitor cell maintenance

Judith A. Blake* and Melanie R. Ziman

ABSTRACT
Pax genes encode a family of transcription factors that orchestrate complex processes of lineage determination in the developing embryo. Their key role is to specify and maintain progenitor cells through use of complex molecular mechanisms such as alternate RNA splice forms and gene activation or inhibition in conjunction with protein co-factors. The significance of Pax genes in development is highlighted by abnormalities that arise from the expression of mutant Pax genes. Here, we review the molecular functions of Pax genes during development and detail the regulatory mechanisms by which they specify and maintain progenitor cells across various tissue lineages. We also discuss mechanistic insights into the roles of Pax genes in regeneration and in adult diseases, including cancer.

KEY WORDS: Pax genes, Embryogenesis, Lineage determination

Introduction
Paired box (Pax) genes encode transcription factors that contain a highly conserved DNA-binding domain called the paired domain (PD, Fig. 1A) and can be considered to be a principle regulator of gene expression. Nine Pax genes (Pax1-Pax9) have been characterised in mammals and the evolutionary conserved paired domain has been identified across phylogenies from insects, to amphibians and birds. In higher vertebrates, PAX proteins are subclassified into groups according to inclusion of an additional DNA-binding homeodomain and/or an octapeptide region, which serves as a binding motif for protein co-factors for potent inhibition of downstream gene transcription (Eberhard et al., 2000) (Fig. 1B); all PAX proteins include a transactivation domain located within the C-terminal amino acids (Underhill, 2012). It is also known that all Pax genes, with the exception of Pax4 and Pax9, produce alternative RNA transcripts (see Table 1). The functional diversity of Pax proteins in vivo is thus linked to the ability to produce alternatively spliced gene products that differ in structure and, consequently, in the binding activity of their paired and homeodomain DNA-binding regions (Underhill, 2012).

Three decades ago, the characterisation and roles of Pax genes in embryonic development began to unfold. Early studies discovered that regulatory gene families such as the Pax family are involved in the sequential compartmentalisation and body patterning of developing organisms; thereafter, studies highlighted a role for Pax genes in the early specification of cell fate and the subsequent morphogenesis of various tissues and organs. Following this, mutational studies of Pax genes confirmed the importance of these regulatory roles in the initiation and progression of development, as well as in disease. The aim of this Primer is to highlight the means by which PAX transcription factors function and to discuss the molecular mechanisms used to determine their tissue-specific activity.

Developmental roles for Pax genes
Studies in animals, together with analyses of human genetics, have revealed important roles for Pax genes (summarised in Table 2) in the development of various organs and tissues, including the thymus (PAX1 and PAX7), vertebrae (PAX1), ear (PAX2 and PAX8), kidney (PAX2), central nervous system (CNS) (PAX2, PAX5, PAX6, PAX7, PAX8 and PAX9), heart vasculature, enteric nervous system, melanocytes, Schwann cells (PAX3 and PAX7), pancreas (PAX4 and PAX6), B lymphocytes (PAX5), eye (PAX6), skeletal muscle (PAX3 and PAX7), thyroid (PAX8) and teeth (PAX 7 and PAX9) (Lechternberg and Ferretti, 1981; Tassabehji et al., 1992; Hoth et al., 1993; Barr et al., 1996; Wallin et al., 1996; Epstein, 1996; St-Onge et al., 1997; Macchia et al., 1998; Nutt and Busslinger, 1999; Eccles and Schimmimenti, 1999; Lang et al., 2000; Stockton et al., 2000; Adham et al., 2005). In order to understand how PAX transcription factors regulate patterns of gene expression and control cellular development, it is essential to determine the mechanisms by which PAX proteins operate, although this has been a major challenge.

Pax6 is one of the most extensively studied members of the Pax family. It not only regulates development of the CNS and is implicated in CNS disease, but also has an important role in the survival of healthy adult brain neurons and their vulnerability to neurodegeneration. Thus, the functional mechanisms of Pax6 action during CNS development have been widely studied and are highlighted here. Pax gene function has also been well characterised during embryonic development of the musculoskeletal system, which is described as a progressive process in which cells undergo lineage determination through the restriction of their cellular potential. In this system, research has aided our understanding of the molecular mechanisms by which Pax genes function to specify cell identity and to regulate this progressive process, which also has important implications for adult regenerative tissue repair and stem cell research. Therefore, somitic development is also the focal point of this Primer. In contrast to somitic cell progenitors, neural crest progenitors acquire a greater developmental potential than the cells from which they are derived and display stem cell characteristics, such as the maintenance of multipotency and the repression of differentiation via genetic and epigenetic mechanisms. The mechanisms by which Pax3 governs differentiation in neural crest precursors is of great interest to developmental biologists and for regenerative medicine; thus, the molecular influence of Pax3 in the cells derived from the neural crest is also discussed here.

PAX6 coordinates specification of the neuroectoderm
PAX6 plays an important role as early as the second week following human conception, when the formation of the CNS commences, as...
Bylund et al., 2003) and is linked to the progression of NE cells to where are in contrast to those observed during mouse NE specification, pluripotent genes (Zhang et al., 2010). Importantly, these findings the NE inductive effects of PAX6A through co-repression of factor 8) and

![Fig. 1. Subclassification of PAX transcription factors according to protein structure.](image)

(A) All PAX proteins contain a transactivation domain and a DNA-binding domain known as the paired domain (PD). The PD consists of PAI and RED subdomains, each of which is composed of three helices in a helix-turn-helix motif. Some PAX family members also contain an additional DNA-binding homeodomain and/or an octapeptide region. (B) PAX proteins are subclassified into four paralogous groups according to full or partial inclusion of the additional DNA-binding homeodomain and the octapeptide region. Adapted, with permission, from Chi and Epstein (Chi and Epstein, 2002).

the neuroectoderm (NE) is specified from the pluripotent epiblast. In humans, there is evidence that PAX6 is necessary for NE specification from embryonic stem cells where it functions to repress the pluripotency genes Oct4, Nanog and Myc and in turn initiate differentiation toward a NE fate. However, PAX6-mediated repression of pluripotency genes is not sufficient for induction of NE differentiation; the PAX6A isoform (Table 1) binds to and induces downstream NE layer genes such as Lhx2 (LIM homeobox protein 2), Six3 (sine oculis-related homeobox 3), Fgf8 (fibroblast growth factor 8) and Wnt5b, while a second isoform, PAX6B, potentiates the NE inductive effects of PAX6A through co-repression of pluripotent genes (Zhang et al., 2010). Importantly, these findings are in contrast to those observed during mouse NE specification, where Pax6 is expressed following the formation of NE cells (Bylund et al., 2003) and is linked to the progression of NE cells to radial glia (Suter et al., 2009), suggesting distinct roles for PAX6 in human versus mouse NE specification.

**PAX-controlled regionalisation of the neural tube**

Pax2 is the earliest Pax gene to be expressed during mouse neurulation, exhibiting expression at embryonic day 7.5 (E7.5) in the neural plate area of the future mid/hindbrain region, where it is essential for formation of the midbrain-hindbrain boundary (MHB), which controls midbrain and cerebellar development (Pfeffer et al., 2002). Subsequently, Pax5 and Pax8 are also expressed in this region, where their temporal sequence of activation is crossregulated by PAX2. In Pax2 mutant embryos, Pax5 expression is absent from the MHB and this loss is linked to loss of PAX-mediated regulation of the MHB-specific enhancer of Pax5 (Pfeffer et al., 2000). Interestingly, Pax2 expression in the MHB is controlled by early and late enhancers, the latter of which is auto- and crossregulated by Pax5 and Pax8, respectively (Pfeffer et al., 2002). These crossregulatory feedback loops are thought to ‘maintain, sharpen and stabilise’ the Pax2 domain at the MHB (Pfeffer et al., 2002).

Around the fourth week of human gestation, the cephalic end of the neural tube forms three brain vesicles, which correspond to the forebrain (telencephalon and diencephalon), midbrain (mesencephalon) and hindbrain (metencephalon and myelencephalon). The formation of these various rudimentary brain structures is brought about by boundary formation and neural progenitor specification. Regionalisation of the neural tube by boundary formation is evident as early as E8 in mouse, when Pax6 and Pax2 transcripts are first detected in the presumptive forebrain and midbrain where their expression corresponds to the establishment of a boundary between the di- and mesencephalic areas (Walther and Gruss, 1991). It has been proposed by Matsunaga et al. (Matsunaga et al., 2000) that Pax6 is induced in the rostral region as Pax2 is induced in the caudal region, giving rise to an overlap of Pax6 and Pax2 expression at the di-mesencephalic boundary. In this region, Pax6 and Pax2 are proposed to repress each other, by indirect and direct molecular mechanisms, until their expression domains are completely segregated, thus forming the di-mesencephalic boundary (Fig. 2). Altered regionalisation, such as that occurring in Pax6 mutant mice, leads to a misexpression of genes that is characteristic of the neighbouring brain region and ultimately a change in the neuronal fate of that region (Mastick et al., 1997; Schwarz et al., 1999). Thus, the direct and indirect effects of Pax gene expression and regionalisation leading to patterning of the brain are tightly controlled.

An example of the series of positive and negative regulatory events involved in early brain regionalisation is seen during specification of the optic tectum, which is linked to the activities of Pax3 and Pax7, and of the transcription factors Meis2 (Meis homeobox 2) and Otx2 (orthodenticle homolog 2). Otx2 expression is a prerequisite for development of all anterior brain structures and is present in the entire neural tube anterior to the MHB (Acampora et al., 1995). Meis2 competes with Groucho co-repressor Tcl4 for binding to Otx2, and formation of Meis2/Otx2 complexes confers tectal specificity in the dorsal midbrain. Meis2, however, autostimulates its expression in the tectal anlage; therefore, an inhibitory mechanism is required to prevent overexpression and faithfully demarcate the prospective tectal area (Agoston and Schulte, 2009). Using chick in ovo electroporation gain-of-function Pax3/7 experiments, it has been reported that tectal expression of Meis2 is balanced by Pax3/7 regulation. Moreover, Meis2 not only associates with Otx2 but interacts with Pax3 and Pax7 in order to
function both as a transcriptional activator of downstream tectum-associated genes and to repress the diencephalic marker Pax6 (Agoston et al., 2012).

PAX6 functions in the developing spinal cord
As regionalisation and specification of the neurons of the developing brain occurs, the remainder of the caudal neural tube forms the spinal cord, in which the distinction of neuronal cell subtypes that will control either sensory input or motor output is controlled by intricate gene regulatory networks. Motoneurons differentiate from progenitor cells via graded sonic hedgehog (Shh) protein signals secreted from the notochord and floorplate. Concentrations of Shh ligands are said to be ‘interpreted’ by progenitor cells so as to initially define their identity and position into four distinct neuronal subtypes within the ventral spinal cord (Ericson et al., 1997). Although a relationship between Shh, Olig2 (oligodendrocyte transcription factor 2), Pax6 and Nkx2.2 (NK2 homeobox 2) has been demonstrated, whereby Pax6 and Nkx2.2 were affected inversely by rising concentrations of Shh (Ericson et al., 1997), this model was challenged in terms of the direct effects of morphogen gradients and was further tuned to incorporate non-graded feedback circuits between Shh, Olig2, Pax6 and Nkx2.2; Nkx2.2 intrinsically strengthens Shh responses by positive feedback, whereas Pax6 is a Shh antagonist (Lek et al., 2010).

Further studies of neuroblast specification demonstrated that the response of neuroblasts to Shh was linked to factors other than transcriptional sensitivity to Shh signals. Using an in vivo reporter, intracellular activity of Gli (a transcriptional effector of Shh signalling) was found to be part of a Pax6-Olig2-Nkx2.2 transcriptional regulatory network (Balaskas et al., 2012). A mathematical model of the network was tested using simulated temporal and spatial changes in Gli activity, and graded gene expression outputs of neuroblasts were shown to be a product of the regulatory ‘logic’ of the Pax6-Olig2-Nkx2.2 network (Balaskas et al., 2012). Thus, the level and duration of Shh signals did not alone control neuroblast specification, as the regulatory network confers robustness and insensitivity to transient changes in the level of Shh signals (Balaskas et al., 2012).

In addition to specifying subsets of neurons in the developing spinal cord, Pax6 also functions to control the balance between neuronal progenitor proliferation and differentiation. Using gain and loss of Pax6 expression experiments in the chick neural tube, it was found that a threshold of Pax6 was crucial for cell cycle exit and neuronal commitment. However, once the neuronal progenitor is committed, Pax6 must be downregulated by neurogenin 2 (Neurog2) to allow differentiation (Bel-Viallar et al., 2007). This characteristic Pax ‘on/off switch’ is said to temporally maintain a precursor in a ‘pre-differentiation’ state and is recapitulated in melanoblast and peripheral glioblast development (Kioussi et al., 1995; Lang et al., 2005).

PAX6-mediated control of neurogenic proliferation and differentiation in the cerebral cortex
In the developing mouse telencephalon, when the neural plate and neural tube consist of a single cell layer of neuroepithelium, Pax6 controls the balance between neural stem cell self-renewal (symmetric cell division) and neurogenesis (asymmetric cell divisions that produce a neuron and a neurogenic progenitor) (Sansom et al. 2009; Asami et al., 2011). Before neurogenesis, neuroepithelial stem cells divide in a symmetric manner to yield progenitors for the tangential growth of the cerebral cortex. Using transcriptome-based analysis to follow changes in Pax6 levels in vivo, it was shown that Pax6 initially regulates neocortical cell cycle progression by direct regulation of Hmgac2 (high mobility group AT-hook 2), Tle1 (transducin-like enhancer of split 1) and Cdk4 (cyclin-dependent kinase 4), while it indirectly controls the expression of cyclins, Hes5 (hairy and enhancer of split 5) and Notch ligands to promote self-renewal. This temporal regulation of self-renewal is then overcome by Pax6-mediated promotion of neurogenesis via Neurog2 and Sox4 (sex-determining region Y, box 4), whereby an increased dose of Pax6 pushes the system towards neurogenesis at the expense of self-renewal (Sansom et al., 2009).

By E10/11, the neuroepithelium transforms into a number of cell layers as neural stem cells lose epithelial features and give rise to fate-restricted radial glia (RG) and basal progenitors (BPs). RG remain confined to the lining of the ventricle (apical layer) where their densely packed cell nuclei undergo mitosis in the ventricular zone. BPs originate from neuroepithelial or RG at the apical surface of the ventricular zone, but retracted their apical cell processes at mitosis and remain at the basal side of the ventricular zone to form the subventricular zone. During neurogenic cell divisions, the orientation of cleavage planes dictates whether the division will be symmetric or asymmetric. Crucial apical and basal constituents (such as those of the adherens junction) are thus either distributed equally to daughter cells or apical constituens are inherited by one daughter cell and basal constituents to the other. An increase in asymmetric divisions of neurogenic progenitors of the neocortex has been demonstrated in Pax6<sup>Sey</sup>/Sey mutant mice (Götz et al., 1998); therefore, the cleavage angles of apical progenitors were assessed in both Pax6<sup>Sey</sup>/Sey mice and a conditional Pax6 knockout model. In both cases, Pax6 disruption significantly altered spindle orientation in apical progenitors at mid-neurogenesis (E14-E16) into oblique and horizontal planes. Alteration of the cleavage plane resulted in the unequal inheritance of apical constituents, which prevented the daughter cell from delaminating in the basal direction and resulted in proliferative daughter cells with radial glial characteristics but division at a sub-apical position. It was concluded that Pax6 regulates several components of apical junction coupling, in particular Spag5 (sperm associated antigen 5), which is involved in the localisation of spindle poles and kinetochorees during cell division (Asami et al., 2011).

The molecular mechanism by which Pax6 achieves either proliferation or neurogenesis in neocortical cells is via distinct functions of its paired domain subdomains. For example, using mice with point mutations in either the PAI or the RED subdomain (Fig. 1) and chromatin immunoprecipitation experiments, it was demonstrated that distinct downstream target genes were preferentially bound by either subdomain in vivo and that the PAI subdomain has a role in decreasing cell mitoses while the RED domain has the opposite effect. It was concluded that the co-activation of both proliferative and anti-proliferative genes acts to fine-tune cell-cycle progression (Walcher et al., 2013). This supposition is supported by the finding that Pax6 directs cortical cell cycle progression in a regionally specific manner (Mi et al., 2013) and that it does so through simultaneous expression of downstream target gene sets through epigenetic regulation of chromatin condensation in interaction with dynamically competitive BAF subunits during the progression of neurogenesis (Tuoc et al., 2013).

Neural crest specification, migration and differentiation: the roles of PAX3 and PAX7
Before the neural tube closes, a ridge of cells known as the neural crest (NC) appears along the margins of the neural folds. Melanocytes, neurons, peripheral glia, mesenchyme, facial
### Table 1. Alternative PAX transcripts

<table>
<thead>
<tr>
<th>Transcription name</th>
<th>Description</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pax1 variant 1</td>
<td>Encodes the longest isoform of Pax1</td>
<td></td>
<td>Burri et al., 1989; Schnitzer et al., 1992</td>
</tr>
<tr>
<td>Pax1 variant 2</td>
<td>Uses an alternate splice site in the 3’ coding region, which results in a frameshift compared with variant 1. Encodes an isoform that has a shorter and distinct C terminus compared with variant 1.</td>
<td></td>
<td>Burri et al., 1989; Smith and Tuan, 1994</td>
</tr>
<tr>
<td>Pax2 variant a</td>
<td>Uses an alternate in-frame splice site in the 3’ coding region compared with variant e (see below) resulting in a shorter protein that has a shorter, distinct C terminus compared with variant e (see below)</td>
<td>Cell differentiation during sensory placode formation (McCarroll et al., 2012); otic specification (Hans et al., 2004)</td>
<td>Eccles et al., 1992; Ward et al., 1994</td>
</tr>
<tr>
<td>Pax2 variant b</td>
<td>Lacks an alternate in-frame exon and uses an alternate splice site in the 3’ coding region compared with variant e (see below), resulting in a protein with a shorter, distinct C-terminus compared with variant e (see below)</td>
<td>Maintenance of the otic placode (Mackereth et al., 2005)</td>
<td>Eccles et al., 1992; Ward et al., 1994</td>
</tr>
<tr>
<td>Pax2 variant c</td>
<td>Has multiple differences in the coding region compared with variant e (see below), one of which results in a translational frameshift. The resulting protein has a distinct C terminus and is shorter than variant e.</td>
<td></td>
<td>Eccles et al., 1992; Ward et al., 1994</td>
</tr>
<tr>
<td>Pax2 variant d</td>
<td>Lacks an alternate in-frame exon compared with variant e (see below). This results in an isoform that is shorter than variant e.</td>
<td></td>
<td>Tavassoli et al., 1997</td>
</tr>
<tr>
<td>Pax2 variant e</td>
<td>This variant encodes the longest isoform</td>
<td></td>
<td>Eccles et al., 1992; Ward et al., 1994</td>
</tr>
<tr>
<td>Pax3a</td>
<td>Differs in the 3’ UTR, includes an alternate segment in the coding region that causes a frameshift and lacks several segments in the 3’ coding region compared with isoform Pax3c. The resulting protein lacks the paired-type homeodomain and has a shorter and distinct C terminus compared with isoform Pax3c.</td>
<td>Negative effects on melanocyte proliferation (Wang et al., 2006)</td>
<td>Tassabehji et al., 1992; Carezani-Gavin et al., 1992</td>
</tr>
<tr>
<td>Pax3b</td>
<td>Differs in the 3’ UTR, includes an alternate segment in the coding region that causes a frameshift and lacks several segments in the 3’ coding region compared with isoform Pax3c. The resulting protein lacks the paired-type homeodomain and has a shorter and distinct C terminus compared with isoform Pax3c.</td>
<td>Negative effects on melanocyte proliferation; reduced migration and accelerated apoptosis (Wang et al., 2006)</td>
<td>Tassabehji et al., 1992; Carezani-Gavin et al., 1992</td>
</tr>
<tr>
<td>Pax3c</td>
<td>The constitutive splice pattern. The resulting protein, PAX3C, is also known as PAX3.</td>
<td>Promotes melanocyte proliferation, migration, transformation and survival (Wang et al., 2006); required for terminal myogenic differentiation (Charytonowicz et al., 2011)</td>
<td>Tassabehji et al., 1992; Wang et al., 2006</td>
</tr>
<tr>
<td>Pax3d</td>
<td>Differs in the 3’ UTR and contains an alternate splice pattern in the 3’ coding region compared with isoform Pax3c. The resulting protein is longer and has a distinct C terminus compared with isoform Pax3c.</td>
<td>Promotes melanocyte proliferation, migration, transformation and survival (Wang et al., 2006); involved in undifferentiated cell maintenance and/or proliferation (Charytonowicz et al., 2011)</td>
<td>Barber et al., 1999; Wang et al., 2006</td>
</tr>
<tr>
<td>Pax3e</td>
<td>Differs in the 3’ UTR and contains an alternate splice pattern in the 3’ coding region compared with isoform Pax3c. The resulting protein is longer and has a distinct C terminus compared with isoform Pax3c.</td>
<td>Reduced melanocyte growth (Wang et al., 2006)</td>
<td>Wang et al., 2006</td>
</tr>
<tr>
<td>Pax3g</td>
<td>Differs in the 3’ UTR and contains an alternate splice pattern in the 3’ coding region compared with isoform Pax3c. The resulting protein is shorter and has a distinct C terminus compared with variant Pax3c.</td>
<td>Melanocyte migration was reduced (Wang et al., 2007)</td>
<td>Wang et al., 2007</td>
</tr>
<tr>
<td>Pax3h</td>
<td>Differs in the 3’ UTR and contains an alternate splice pattern in the 3’ coding region compared with isoform Pax3c. The resulting protein is shorter and has a distinct C terminus compared with isoform Pax3c.</td>
<td>Increased melanocyte proliferation, migration, survival and transformation (Wang et al., 2006)</td>
<td>Wang et al., 2006</td>
</tr>
<tr>
<td>Pax5</td>
<td>No data available on NCBI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pax6 variant 1</td>
<td>Also known as Pax6(-5a). Represents the longest transcript. Variants 1, 3, 6 and 7 encode the same isoform (a).</td>
<td>Neurogenesis, proliferation, regionalisation and boundary formation</td>
<td>Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992</td>
</tr>
<tr>
<td>Pax6 variant 2</td>
<td>Also known as Pax6(+5a). Differs in the 5’ UTR and includes an insertion of 14 amino acids (exon 5a) into the paired domain compared with variant 1. Variants 2, 4 and 5 encode the same isoform (b), which is shorter than isoform a.</td>
<td>Overexpression leads to proliferation</td>
<td>Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992</td>
</tr>
<tr>
<td>Pax6 variant 3</td>
<td>Differs in the 5’ UTR compared to variant 1. Variants 1, 3, 6 and 7 encode the same isoform (a).</td>
<td>Neurogenesis, proliferation, regionalisation and boundary formation</td>
<td>Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992</td>
</tr>
<tr>
<td>Pax6 variant 4</td>
<td>Differs in the 5’ UTR and includes an alternate exon in the coding region that maintains the reading frame compared with variant 1. Variants 2, 4 and 5 encode the same isoform (b), which is shorter than isoform a.</td>
<td>Overexpression leads to proliferation</td>
<td>Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992</td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Transcript name</th>
<th>Description</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pax6 variant 5</td>
<td>Differs in the 5' UTR and includes an alternate exon in the coding region that maintains the reading frame compared with variant 1. Variants 2, 4 and 5 encode the same isoform (b), which is shorter than isoform a.</td>
<td>Overexpression leads to proliferation</td>
<td>Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992</td>
</tr>
<tr>
<td>Pax6 variant 6</td>
<td>Differs in the 5' UTR compared with variant 1. Variants 1, 3, 6 and 7 encode the same isoform (a).</td>
<td>Neurogenesis, proliferation, regionalisation and boundary formation</td>
<td>Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992</td>
</tr>
<tr>
<td>Pax6 variant 7</td>
<td>Differs in the 5' UTR compared with variant 1. Variants 1, 3, 6 and 7 encode the same isoform (a).</td>
<td>Neurogenesis, proliferation, regionalisation and boundary formation</td>
<td>Vorobyov et al., 1997; Barr et al., 1999</td>
</tr>
<tr>
<td>Pax7 variant 1</td>
<td>Encodes the longest Pax7 isoform</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pax7 variant 2</td>
<td>Uses an alternate in-frame splice site compared with variant 1. The resulting isoform has the same N and C termini but is two amino acids shorter than variant 1.</td>
<td>A more potent transactivator of Cntfr (White and Ziman, 2008);</td>
<td></td>
</tr>
<tr>
<td>Pax7 variant 3</td>
<td>Differs in the 3' UTR and coding sequence compared with variant 1. The resulting isoform has a shorter and distinct C terminus compared with variant 1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pax8a</td>
<td>Encodes the longest Pax8 isoform</td>
<td>Activating domain encoded by exons 10 and 11</td>
<td>Poleev et al., 1995</td>
</tr>
<tr>
<td>Pax8b</td>
<td>Uses an alternate splice site in the 3' coding region compared with isoform Pax8a that results in a frameshift and an early stop codon. It encodes an isoform that has a shorter and distinct C terminus compared to Pax8a.</td>
<td>Antagonistic role to the activating domain</td>
<td>Poleev et al., 1995</td>
</tr>
<tr>
<td>Pax8d</td>
<td>Lacks two alternate exons compared with isoform Pax8a that results in a frameshift and an early stop codon. The encoded isoform is shorter and has a distinct C terminus compared with Pax8a.</td>
<td></td>
<td>Poleev et al., 1995</td>
</tr>
<tr>
<td>Pax8e</td>
<td>Lacks three alternate exons compared to isoform Pax8a that results in a frameshift and an early stop codon. The encoded isoform is shorter and has a distinct C terminus compared with Pax8a.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pax9</td>
<td>No data available on NCBI</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ctnfr, ciliary neurotrophic factor receptor.

cartilage and bone originate from NC cells (Le Douarin et al., 2008), and Pax3 and Pax7 play key roles in their specification, migration and differentiation into the various tissue lineages and regions. Initially, cells of the neural plate border respond to signals from the neural plate that upregulate Pax3, Pax7, Zic1 (zinc finger protein of the cerebellum 1), and Msx1 and Msx2, early markers of the NC lineage in mice and genes required for NC induction (Sato et al., 2005; Monsoro-Burq et al., 2005; Basch et al., 2006). Notably, the role of Pax3 and Pax7 during specification of the NC lineage is distinct in chick and Xenopus when compared with mouse (Sato et al., 2005; Basch and Bronner-Fraser, 2006); in mice, Pax3 and Pax7 function to upregulate the downstream NC specifier genes Snail, Foxd3 (forkhead box D3) and Sox genes, which are said to then ‘imbue’ the cell to become a competent NC cell (Bronner, 2012).

Although the structures of their DNA-binding domains are similar (Vorobyov et al., 1997), differences in Pax3 and Pax7 function and regulation during NC development have been identified. Using Pax7-cre/reporter mice, Pax7-expressing precursors were noted to be distinct from Pax3-expressing NC precursors, and principally contribute to the cranial NC derivatives of the fronton parietal bones of the skull, meninges, teeth, trigeminal ganglia, whisker follicles, olfactory epithelium and cartilage of the nasal septum (Engleka et al., 2005; Murdoch et al., 2012). Often thought to act in a redundant manner in regions of overlapping expression, the two proteins also exhibit differences in their regulatory mechanisms. The Pax7 transactivation domain, for example, is sumoylated, whereas the Pax3 transactivation domain is not. This post-translational sumoylation of Pax7 has been shown to be essential for early NC formation (Luan et al., 2013).

Pax3 has a crucial role in the induction and specification of NC cells. Using pluripotent cells of the Xenopus blastula in vitro, it was shown that electroporation of a Pax3/Zic1 construct prior to neurulation induced the early NC specifiers snail1, sox8 and myc in cells that went on to undergo an epithelial-to-mesenchymal transition, detach from the explant and migrate in vivo following xenotransplantation into a host embryo. Pax3/Zic1-induced and grafted NC cells followed normal NC migration paths and were seen frequently to develop into pigmented melanocytes and chondrocyte cells (Milet et al., 2013). Pax3 also regulates Hes1 (hair and enhancer of split 1; which is involved in neural stem cell maintenance), Neurog2 (which is involved in NC neurogenesis) and Tgfβ2 (transforming growth factor β2) (which in turn represses Pax3 promoter activity on Hes1 and Neurog2) (Theriault et al., 2005; Barr et al., 1999; Nakazaki et al., 2008; Medic and Ziman, 2010). Using mice bred for a double heterozygous Pax3 and Tgfβ2 phenotype, Nakazaki et al. (Nakazaki et al., 2009) demonstrated that the Tgfβ2+/− Pax+/+ phenotype of open neural tube and bifid spine was reversed in 85% of animals following the loss of one Pax3 allele (Tgfβ2+/− Pax+/−). Results were attributed to the opposing roles of Pax3 and Tgfβ2 in the regulation of Hes1, Neurog2 and Sox9, as seen in earlier studies (Mayanil et al., 2001; Zavadil et al., 2001). Following this, a model was described in which the regulatory network between Pax3, Tgfβ2, Hes1, Neurog2 and Sox9 functions to maintain NC cells in an undifferentiated state prior to migration while ensuring progenitor cell identity is specified through appropriate timing of Hes1, Neurog2 and Sox9 expression (Nakazaki et al., 2009). Nakazaki et al. also discussed a possible role for Pax3 and Tgfβ2 in remodelling of the extracellular matrix to facilitate NC cell migration (Nakazaki et al., 2009); however, the continuing...
Table 2. PAX mutations and associated phenotypes

<table>
<thead>
<tr>
<th>Name/species</th>
<th>Mutation</th>
<th>Pathology</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undulated/mouse</td>
<td>G15S (paired-domain) Pax1</td>
<td>Abnormalities in the sternum and parts of the vertebral column in heterozygotes</td>
<td>Ballying et al., 1988</td>
</tr>
<tr>
<td>Scoliosis/mouse</td>
<td>2.0 kb and 4.5 kb deletions exons 1 to 4, respectively, in Pax1</td>
<td>Homozygous mice show lumbar scoliosis and a kinked tail</td>
<td>Adham et al., 2005</td>
</tr>
<tr>
<td>Papillorenal syndrome/human</td>
<td>Heterogenous mutations to Pax2</td>
<td>Ocular and renal anomalies, vesicoureteral reflux, high frequency hearing loss, central nervous system anomalies and/or genital anomalies</td>
<td>Schimmelfent et al., 1997; Eccles and Schimmenti, 1999; Devriendt et al., 1998; Desvrieux et al., 2000; Nishimoto et al., 2001; Hijigashie et al., 2005; Bower et al., 2012</td>
</tr>
<tr>
<td>Craniofacial-deafness-hand syndrome/human</td>
<td>Asn47-to-lys; asn47-to-his (paired domain) Pax3</td>
<td>Flat facial profile, hypertelorism, hypoplastic nose with slidiike nares, sensorineural hearing loss. small maxilla, absent or small nasal bones and ulnar deviation of the hands</td>
<td>Hoth et al., 1993; Asher et al., 1996</td>
</tr>
<tr>
<td>Rhabdomyosarcoma 2, alveolar/human</td>
<td>Translocation of the Pax3 gene on chromosome 2 or Pax7 gene on chromosome 1 with the FKHR/FOXO1A gene on chromosome 13</td>
<td>Childhood sarcoma</td>
<td>Douglass et al., 1987; Wang-Wu et al., 1988; Hayashi et al., 1988; Barr et al., 1991; Barr et al., 1993; Fredericks et al., 1995</td>
</tr>
<tr>
<td>Waardenburg syndrome, type 1/human</td>
<td>Heterogenous mutations in Pax3</td>
<td>Pigmentary abnormalities of the hair, skin and eyes; congenital sensorineural hearing loss; dystopia canthorum; lateral displacement of the ocular inner canthi</td>
<td>Tassabehji et al., 1992; Baldwin et al., 1992; Ishikiniyama, 1993; Baldwin et al., 1995</td>
</tr>
<tr>
<td>Waardenburg syndrome, type 3/human</td>
<td>Heterogenous mutations in Pax3</td>
<td>Pigmentary abnormalities of the hair, skin and eyes; congenital sensorineural hearing loss; dystopia canthorum; lateral displacement of the ocular inner canthi and upper limb abnormalities</td>
<td>Hoth et al., 1993; Zlototoga, 1995; Tekin et al., 2001; Wollink et al., 2003</td>
</tr>
<tr>
<td>Splotch/mouse</td>
<td>A-to-T transversion of the AG splice acceptor of intron 3 of Pax3</td>
<td>Neural tube, neural crest, severe limb and trunk muscle defects</td>
<td>Epstein et al., 1991</td>
</tr>
<tr>
<td>Splotch-delayed/mouse</td>
<td>G9R (paired domain) Pax3</td>
<td>Heterozygous animals have pigmentation defects with occasional neural tube defects; homozygous animals have spina bifida with or without exencephaly, spinal ganglia abnormalities and delays in posterior neuropore closure and neural crest cell emigration</td>
<td>Yang and Trasler, 1991</td>
</tr>
<tr>
<td>Ketosis-prone diabetes mellitus/human</td>
<td>Ethnic-specific gene variants in PAX4</td>
<td>Severe hyperglycemia and ketosis</td>
<td>Sobngwi and Gautier, 2002; Mauvais-Jarvis et al., 2004</td>
</tr>
<tr>
<td>Diabetes mellitus, type 2/human</td>
<td>Heterogenous mutations of PAX4</td>
<td>Weight loss, fatigue, polydipsia, polyuria and hyperglycemia</td>
<td>Shimajiri, 2001</td>
</tr>
<tr>
<td>Lymphoplasacytoid lymphoma/human</td>
<td>Haploinsufficiency of PAX5</td>
<td>Leukaemia</td>
<td>Glaser et al., 1990; Hill et al., 1991; Matsu et al., 1993; Ramaesh et al., 2003; Ramasah et al., 2006</td>
</tr>
<tr>
<td>Small eye/mouse</td>
<td>Mutation in Pax6</td>
<td>Anidria and corneal abnormalities</td>
<td>Glaser et al., 1990; Hill et al., 1991; Matsu et al., 1993; Ramaesh et al., 2003; Ramasah et al., 2006</td>
</tr>
<tr>
<td>Aniridia/human</td>
<td>Mutation in PAX6</td>
<td>Noticeable iris hypoplasia</td>
<td>Shaw et al., 1960; Elsas et al., 1977; Glaser et al., 1994; Sisodiya et al., 2001</td>
</tr>
<tr>
<td>Coloboma of optic nerve/human</td>
<td>Mutation in PAX6</td>
<td>Detachment of the macula</td>
<td>Jonas and Freisler, 1997; Hornby et al., 2000; Azuma et al., 2003</td>
</tr>
<tr>
<td>Foveal hyperplasia/human</td>
<td>Mutation in PAX6</td>
<td>Subnormal visual acuity and congenital nystagmus</td>
<td>O'Donnell and Pappas, 1982; Hanson et al., 1999; O'Donnell and Pappas, 1982; Curran and Robb, 1976; Oliver et al., 1987; Azuma et al., 1996; Hanson et al., 1999</td>
</tr>
<tr>
<td>Gillespie syndrome/human</td>
<td>Mutation in PAX6</td>
<td>Anidria, cerebellar ataxia and mental retardation</td>
<td>Gillespie, 1965; Sarsfield, 1971; Crawfurd et al., 1979; Lechtenberg and Ferretti, 1981; Wigit et al., 1988; François et al., 1984; Nelson et al., 1997; Verhulst et al., 1993; Ticho et al., 2006; Graziano et al., 2007; Kivlin et al., 1986; Pearce et al., 1995; Hackenbruch et al., 1975; Azuma et al., 2003</td>
</tr>
<tr>
<td>Keratitis/human</td>
<td>Mutation in PAX6</td>
<td>Childhood corneal clouding</td>
<td>Girgis et al., 1993; Fredericks et al., 1995</td>
</tr>
<tr>
<td>Optic nerve hypoplasia/human</td>
<td>Mutation in PAX6</td>
<td>Bilateral optic nerve hypoplasia, poor visual acuity and wandering movements of the eyes</td>
<td>Hackenbruch et al., 1975; Azuma et al., 2003</td>
</tr>
<tr>
<td>Peters anomaly/human</td>
<td>Mutation in PAX6</td>
<td>Central corneal leukemia, absence of the posterior corneal stroma and lenticular attachments to the central aspect of the posterior cornea</td>
<td>Hanson et al., 1994; Yang et al., 2004</td>
</tr>
<tr>
<td>Hypothyroism, congenital, due to thyroid dysgenesis or hypoplasia/human</td>
<td>Heterogenous mutations of PAX8</td>
<td>Congenital hypothyroism, ectopic thyroid gland, athyreotic cretinism</td>
<td>Macchia et al., 1998; Vilain et al., 2001; Congdon et al., 2001; Meese et al., 2004</td>
</tr>
<tr>
<td>Tooth agenesis, selective, 3/human</td>
<td>Heterogenous mutations of PAX9</td>
<td>Oligodontia and hypodontia</td>
<td>Stockton et al., 2000; Das et al., 2002; Frazier-Bowers et al., 2002; Lammi et al., 2003; Mostowska et al., 2006; Kapadia et al., 2006</td>
</tr>
<tr>
<td>Pax9-neo/mouse</td>
<td>Hypomorphic Pax9 allele</td>
<td>Oligodontia</td>
<td>Kist et al., 2005</td>
</tr>
</tbody>
</table>
uncertainty about Pax3 requirement for NC migration (Li et al., 1999; Conway et al., 2000; Kwang et al., 2002; Chan et al., 2004) has been addressed recently by looking at the role of Pax3 in migratory cardiac NC cells using the Pax3 mutant Splotch mouse models, all six of which have different mutations in Pax3 (Table 2). In all Splotch mice, cardiac NC cells fail to undergo proliferative expansion prior to migration due to defects intrinsic to the NC cell. The resulting reduced number of migratory cardiac NC cells in the outflow region of the heart leads to hypoplasia of the posterior pharyngeal arch arteries, vessel destabilisation, lack of septation, an aortic arch at the cervical level and loss of ductus arteriosus (Conway et al., 1997). Importantly, recent studies of a conditional allele with targeted lineage-restriction deletion of Pax3 around E8.0 revealed that Pax3 plays an essential role in early NC precursor formation and specification, but is not required for progeny migration or morphogenesis of the outflow tract of the heart (Olaopa et al., 2011).

It is still not known whether NC cells are lineage restricted before or after emigration from the neuraxis. In mice, premigratory and migratory NC cells have been identified as multipotent using in vivo single cell dye injection and tracking methods (Serbedzija et al., 1994); avian NC cells, however, appear fate restricted just after emergence from the neuraxis (Henion and Weston, 1997) where timing of emigration correlates with localisation to particular pathways and, ultimately, to fate restriction (Krispin et al., 2010). The fate restriction of NC cells is particularly intriguing in relation to Pax3 expression and Schwann cell or melanocyte development. For example, the dorsolateral region between the dermomyotome and the ectoderm is populated by cells that eventually target the skin, and these ‘specified’ melanoblasts express Mitf (microphthalmia-associated transcription factor), a downstream target of PAX3 (Lang et al., 2005). Mitf is known to be necessary for the survival and proliferation of melanoblasts at this stage of development (Opdecamp et al., 1997; Hornyak et al., 2001) and largely controls the melanocytic gene program. Studies have shown that although Pax3 initiates Mitf expression, it functions at the same time to prevent MITF from activating melanocyte genes until external stimuli in the target skin relieve PAX3 repressive effects (Lang et al., 2005). Thus, although lineage-specific transcription factors such as MITF prime the fate of a cell from early stages of NC emigration, migratory cells are inhibited from differentiation by temporal and environmental effects (Basch et al., 2006; Bronner, 2012). This is the melanocytic example of the ‘on-off’ Pax switch, or capacitor, so eloquently described by Lang et al. (Lang et al., 2005).

In an earlier wave of NC migration, at E11 in mouse, NC progeny also migrate in ventral pathways along developing axons prior to acquisition of either a Schwann or melanocyte fate (Jessen and Mirsky, 2005; Adameyko et al., 2009; Adameyko and Lallemand, 2010; Ernfors, 2010). These bi-potent NC derivatives are termed precursor Schwann/melanocyte (PSM) cells and their fate is largely controlled by association with axonally secreted neuregulin (for adoption of glial fate) or insulin-like growth factor 1 (IGF1) and platelet-derived growth factor (PDGF) (for melanocyte specification) (Adameyko et al., 2009; Adameyko and Lallemand, 2010). As PSM cells migrate along established β-neuregulin 1-secreting axon tracts, their maintenance is regulated by Sox2 and Sox10 (Britsch et al., 2001; Le et al., 2005) and Pax3 (Blanchard et al., 1996; Kioussi et al., 1995). Pax3 is a known survival factor for PSM cells during this mitotic and chemotactic stage; when homozygous Splotch mice perish at E13.5, PSM cells cannot be detected (Franz, 1990) and mice homozygous for the Splotch-delayed allele (Table 2) (which survive until E18.5) contain few PSM cells, all of which perish by E15.5 (Moase and Trasler, 1990).

The transition from PSM cell to immature Schwann cell (ISC) or to melanoblast occurs between E12 and E16. During this time, PSM cells penetrate between axons and fated ISCs begin to engage in radial axonal sorting, a period of extensive mitotic and apoptotic activity that results in the requisite ratio of glial cells to axons (Yu et al., 2005). At the same time, some PSM cells are driven toward a melanoblast fate due to increasing levels of IGF1 and PDGF, and to inhibition of axonal contact (Adameyko and Lallemand, 2010) (Fig. 3). It is notable that Pax3 expression is downregulated in this pathway from E13.5 to E18.5 (Kioussi et al., 1995) as in vitro
studies have shown that Pax3 induces proliferation and inhibits apoptosis of Schwann cells (Doddrell et al., 2012) and also has a principal role in the regulation of dorsolateral melanocyte differentiation (Lang et al., 2005). Downregulation of Pax3 correlates with Oct6 and Krox20 (Egr2; early growth response 2) upregulation (which occurs at E17-P3) in promyelinating Schwann cells (Blanchard et al., 1996) and supports the supposition that Pax3 inhibits the myelination gene program (Kioussi et al., 1995; Doddrell et al., 2012). It is certainly intriguing to consider possible Pax3, Sox10, Mitf, Nrg1 (neuregulin 1), Igf1 and Pdgf gene regulatory networks that function in the specification of ISC's versus melanoblasts at this stage. Moreover, further study is required to compare and contrast the role of Pax3 in the development of Schwann cells and melanoblasts in the ventral NC pathway as several authors have proposed a link between these progenitors and cutaneous melanoma (Hoerter et al., 2012; Cramer and Fesyuk, 2012).

**Morphogenesis of the vertebral column and skeletal musculature**

Toward the end of the second week after human conception, tissue masses known as somites occupy the entire length of the trunk on both lateral aspects of the neural tube. These then become progressively organised into the vertebral column and skeletal muscle, which are characteristic of the segmented vertebrate. The somites, which are derived from paraxial mesoderm, are initially pseudostratified epithelial progenitor cells (Nitzen and Kalcheim, 2013). Cells of the ventromedial part of the somite de-epithelialise, migrate and condense as sclerotome near the notochord and contribute to vertebral bodies, spinal pedicles, neural arch and costal processes (Nitzen and Kalcheim, 2013). Sclerotomal expression of Pax1 and Pax9 is dependent upon Shh secretion from the notochord, and evidence supports the fact that Pax1 is necessary for the specification of ventral sclerotome cells that accumulate prior to chondrogenesis (Chirst et al., 2000). In these cells, Pax1 and Pax9 are required to maintain a high rate of proliferation, thus enabling the precursor pool to reach a critical size and proceed into sclerotome-specific developmental programs (Peters et al., 1999).

Medially, somitic epithelial cells generate myocytes, which dissociate to form a rostral/caudal scaffold of nascent muscle fibres adjacent to the developing dorsal dermamyotome (DM). The DM retains its epithelial characteristic, which consists of multipotent progenitors that will generate myogenic, endothelial and dermal cells (Ben-Yair and Kalcheim, 2008). The DM elongates dorsomedially and ventrolaterally with a secondary generation of DM-derived myocytes that delaminate and intercalate among previously scaffolded muscle fibres to colonise the developing epaxial and hypaxial myotome (Nitzen and Kalcheim, 2013). During this somitic patterning phase, Pax3 is expressed in the presomatic mesoderm and throughout the early somite (Goulding et al., 1991).

As the dermomyotome matures, Pax3 becomes restricted to the epaxial and hypaxial lips (Galli et al., 2008), and, in epaxial cells, it

---

**Fig. 3. Development of Schwann cells and melanocytes from neural crest cells.** Melanoblasts diverge from bipotent precursors around the time that radial axonal sorting occurs among immature Schwann cells (E12-E16 indicated by the blue window) and is linked to a lack of access to nerve-secreted growth factors. The divergent specification of immature Schwann cell and melanoblast occurs during a period marked by an absence of Pax3 expression (E13.5-E18 indicated by the red window) (Kioussi et al., 1995). The developmental time scale is indicated along the bottom. Genes or proteins expressed in each cell type are also indicated. DCT, dopachrome tautomerase; ERBB3, V-ERB-B2 avian erythroblastic leukemia viral oncogene homolog 3; GFAP, glial fibrillary associated protein; IGF, insulin-like growth factor; MITF, microphthalmia-associated transcription factor; KROX20, early growth response 2 (EGR2 – Mouse Genome Informatics); MBP, myelin basic protein; OCT6, POU domain, class 3, transcription factor 1; p75NGFR, nerve growth factor receptor; P0, myelin protein zero; PAX, paired homeobox; PDGF, platelet-derived growth factor; S100, S100 calcium-binding protein; SOX, sex-determining region Y.
is thought to upregulate the myogenic regulatory factor Myf5 (myogenic factor 5) through synergy with ZIC1 and GLI2 on the Myf5 epaxial somite enhancer (Himeda et al., 2013).

In the limbs, Pax3+ progenitors delaminate from the hypaxial dermamyotome and migrate into the developing limbs to provide a pool of progenitors for subsequent myogenesis. In these cells, Pax3 regulates the hepatocyte growth factor receptor Met, which is necessary for hypaxial dermalization and migration (Dietrich, 1999); in the limbs, postnatal myogenesis is induced by Pax3 via alternate Myf5 regulatory enhancers (Bajard et al., 2006). In Pax3 mutant embryos, there are abnormalities in segmentation and loss of epaxial and hypaxial dermomyotome with consequent myotome malformation and loss of trunk and limb musculature (Goulding et al., 1993; Tremblay et al., 1998; Schubert et al., 2001).

Pax7 is also expressed in early epithelial somites, principally within the central ‘sheet’ of DM cells; at this stage, Pax7 expression is inversely correlated to myogenic differentiation (Galli et al., 2008). When the final epithelial somite dissociates, a perpendicularly planar shift of cell division occurs in the central sheet concurrent with generation of dermal and myoblast progenitors; here, Pax7+ myoblast progenitors undergo asymmetric division and delamination results in the production of a basal Pax7+ daughter cell that is directed into the developing myotome (Ben-Yair et al., 2011). It is unknown whether this final DM dissociation produces multipotent cells that are specified upon translocation to the dermis or myotome, or whether there is an asymmetric allocation of cell fate determinants to daughter cells.

As development proceeds, myotomal Pax3+ Pax7+ cells retain a progenitor state to become a resident population necessary for skeletal muscle growth (Buckingham and Relaix, 2007). In these myogenic stem cells, the expression of Pax3 and Pax7 is negatively correlated to that of Myod1, a myocyte marker (Galli et al., 2008). During embryonic myogenesis, the balance between myogenic stem cell self-renewal and differentiation is promoted by Notch or FGF, respectively, where the latter signals myogenesis via upregulation of Fgfr4 (fibroblast growth factor receptor 4) by Pax3 (Lagha et al., 2008). Postnatal Pax3+ Pax7+ offspring either enter the myogenic program through upregulation of the myogenic regulatory factors Myf5 and MyoD1 or retain a Pax3+ Pax7+ Mrf (myelin regulatory factor; Myrf – Mouse Genome Informatics) phenotype and align with nascent myotubes to become resident adult stem cells, known as satellite cells (Relaix et al., 2005). Pax7 mutant mice do not have embryonic skeletal muscle defects; however, Pax7 is required for the postnatal survival of satellite cells and hence adult muscle regeneration (Seale et al., 2000; von Maltzahn et al., 2013). In adult myogenesis, Pax7 inactivation during satellite cell proliferation results in loss of satellite cells and reduced heterochromatin condensation in the rare satellite cells that survive (Günter et al., 2013). Pax3 is also expressed in adult satellite cells (Buckingham et al., 2003) and although its role in these cells remains unknown, Soleimani et al. (Soleimani et al., 2012) have described alternate affinities for paired box- and homeobox-binding motifs that are thought to result in alternate Pax7 and Pax3 target gene regulation in adult satellite cells.

Finally, in the trunk, a fourth somitic compartment, known as the syndetome, originates from the dorsolateral edge of the sclerotome as interactions between skeletal muscle and cartilage lead to specification of tendon progenitors. In this region of sclerotomal condensation, both Pax1 and Pax9 are downregulated once chondrogenesis is initiated (Christ et al., 2000; Brand-Saberi and Christ, 2000). This relieves repression of scleraxis (Scx), the de novo expression of which leads to tendon specification in the cells that adjoin skeletal muscle and chondrogenic tissue (Brent et al., 2003) (Fig. 4).

**Pax genes in tissue regeneration and disease states**

The important roles of Pax genes in development underscores their function in adult tissue regeneration and the repercussions of their aberrant loss, overexpression, re-expression or persistent expression in association with pathology (Table 2) and, in particular, cancer (Li and Eccles, 2012). The Pax functions outlined thus far lend credence to the hypothesis that perturbed Pax-expressing cells ‘hijack’ associative signalling pathways and/or transcription networks, leading to uncontrolled growth and survival. As we discuss below, Pax genes continue to function in the adult organism with a principal role in the regulation of lineage specification and maintenance of a stem-like state in progenitor cells. It is important to realise, however, that the mechanisms of Pax action in adult stem and progenitor cells does not fully recapitulate developmental programs, as fate conversion of adult stem cells is also tightly linked to the stem cell niche environment and to other intrinsic mechanisms (Ninkovic et al., 2013).

**Aberrant Pax gene expression: implications for regeneration and disease**

Childhood alveolar rhabdomyosarcoma (ARMS), a malignant soft tissue tumour that occurs in adolescents and young adults, is one example in which aberrant Pax gene expression leads to cellular oncogenic transformation. ARMS cells express a gene fusion resulting from a t(2;13) or t(1;13) translocation of the Pax3 or Pax7 PD- and HD-encoding regions, respectively, with the transactivation domain of the homeotic gene forkhead box O1 (FOXO1, also known as FKHR and FOXO1A). The resulting Pax3/FOXO1 fusion protein exhibits a greater than 100-fold gain-of-function effect on Pax3 downstream target genes and a dominant-negative effect on wild-type Pax3 expression (Bennicelli et al., 1996). Cellular pathways and mechanisms affected by aberrant Pax:FOXO1 expression include MET signalling (which stimulates cell cycle following postnatal muscle injury), FGF receptor-4- and IGF receptor-mediated growth, and chromatin remodelling, which allows activation of MYOD1 target genes (Keller and Guttridge, 2013).

As a large percentage of paediatric rhabdomyosarcomas arise after the first year of life, muscle satellite cells have been proposed as the cell of origin for ARMS. However, the consequences of late embryonic activation of Pax3:FKhr using a conditional Pax3:FKhr knock-in allele demonstrated that pre- and postnatal Pax3:FKhr expression in satellite cells does not lead directly to ARMS (Keller et al., 2004). The rhabdomyoblast, a signature multicellular cell type seen in ARMS, has also been hypothesised as the transformed progeny of an activated satellite cell or mesenchymal stem cell that contributes to skeletal muscle regeneration and, through several Pax3-associated mechanisms, has been inhibited from differentiation (Relaix et al., 2005; Roeb et al., 2007; Charytonowicz et al., 2009). The cell of origin for ARMS thus remains to be clearly identified.

An emerging concept is that Pax proteins have a role in the epigenetic determination of active or silenced regions of DNA (Dressler, 2011). In satellite cells, lymphoid progenitors and HEK 293 cells, Pax7, Pax5 and Pax2, respectively, have been shown to recruit histone methyltransferase complexes to the promoter regions (Patel et al., 2007) of genes such as Myf5 and Myod1 (McKinnell et al., 2008), where the Pax transactivation domain links to methyltransferase complexes by an interacting protein (Cho et al., 2003; Fang et al., 2009; Diao et al., 2012). Epigenetic
repressive imprints have also been shown to be initiated by PAX proteins through the recruitment of groucho-related gene product 4 (GRG4) to specify a region of chromatin for silencing; in melanoblasts, lymphoid progenitors and HEK 293 cells, PAX-mediated gene repression is associated with the recruitment of GRG4, with both proteins interacting cooperatively for resultant transcriptional repression (Eberhard et al., 2000; Lang et al., 2005; Patel et al., 2013). Thus, aberrant PAX expression may fuel oncogenesis through either forced epigenetic activation or repression of downstream target genes, or through a loss of such PAX controlled epigenetic modifications.

Cell specification and fate restriction have also been linked to the compartmentalisation of the genome into active and inactive domains, such that key developmental genes such as PAX are stably silenced in differentiated cells. This epigenetic tenet must be reconsidered, however, in the light of the PAX re-expression and re-repression that occurs with some forms of homeostatic injury repair. For example, a fundamental characteristic of peripheral nerve regeneration is the ability of adult myelinating Schwann cells to revert from a postmitotic differentiated state into the cell cycle and back. In injured nerves, as Schwann cells become demyelinated, Pax3 is upregulated along with other immature Schwann cell genes (Kioussi et al., 1995). Once regenerated axons reach their target tissue, Pax3 levels peak as the progeny of Schwann cells begin to reproduce myelin and, when the myelinogenic program is well under way, Pax3 is downregulated (Kioussi et al., 1995). Pax3 in regenerative Schwann cells, as in embryogenesis, is thus thought to oppose myelination and withdrawal from the cell cycle (Doddrell et al., 2012).

A similar phenotypic regression is seen in adult tubular cells when Pax2 is re-activated during kidney repair. During normal nephron development, Pax2 drives a pivotal mesenchymal-to-epithelial transition event in which ureteric bud cells induce stem cells of the metanephric mesenchyme to transition into epithelial cells (Dziarmaga et al., 2003). However, in renal fibrosis, which is a common manifestation of chronic kidney disease (Liu, 2004), adult tubular cells lose their epithelial characteristics and readopt a mesenchymal (stem cell) fate in order to be involved with injury repair. Studies have shown that Pax2 and Wilms’ tumor 1 gene (Wt1) are both required for mesenchymal-to-epithelial transition and are re-expressed in animal and cell models of tubular epithelial-to-mesenchymal transition during acute and chronic injury (Huang et al., 2012). This so-called ‘atavistic’ phenotypic transition, which triggers the genetic and cellular processes involved with the loss of epithelial phenotype and reacquisition of an induced mesenchymal
stem-like status during repair, is said to mimic, in reverse, the process of mesenchymal-to-epithelial transition in nephrogenesis (Jiang et al., 2013). With both of these examples, it may be queried whether Pax gene re-activation involves some reinstallation of earlier chromatin modifications, in reverse, as dedifferentiated cells re-enter the cell cycle to give rise to progeny. Bivalent chromatin domains are known to be clustered around developmental genes such as Pax in embryonic cells to confer robust gene repression while poising the gene for activation. This bivalency consists of large regions of H3 lysine 27 methylation (and hence compacted chromatin) that harbour smaller regions of H3 lysine 4 methylation (which positively regulates transcription through recruitment of nucleosome remodelling enzymes and histone acetylases). In embryonic stem cells, bivalent chromatin domains resolve progressively during cellular differentiation into broad marked regions of either enriched Lys27 or Lys4 chromatin methylation (Bernstein et al., 2006). Thus, it would be interesting to investigate Pax chromatin modifications in kidney tubules and Schwann cells during responses that involve dedifferentiation of injured cells and redifferentiation of their progeny. An understanding of this Pax epigenetic switching during homeostatic tissue repair may shed light on the aberrant processes of repair that may occur in chronic kidney disease and neurofibromatosis (Pongpudpunth et al., 2010). In addition, such an understanding could be applied to the therapeutic control of PAX gain of function in ARMS and other cancers.

A role for Pax genes in cancer stem cells?

As Pax genes are responsible for the maintenance of a stem-like phenotype, it is possible that they may be associated with maintenance of the cancer stem cell phenotype, a key characteristic of which is the capacity for the oncogenic cell to self-renew. Cancer development is clearly associated with changes in the molecular mechanisms that regulate stem cell differentiation (Perrotti et al., 2010; Nguyen et al., 2012) that are thought inherited by amplified progeny. In 2008, Rudnicki et al. (Rudnicki et al., 2008), using a Myf5-Cre knock in allele, revealed that a novel subset of Pax7-expressing satellite cells does not express Myf5 and that these cells give rise to Pax7+ Myf5+ cells through basal-apical asymmetric cell divisions. Isolation and transplantation of these cells into muscle revealed that they underwent differentiation while Pax7+ Myf5+ cells contributed to the satellite stem cell reservoir.

A subsequent study using transgenic Tg:Pax7-nGFP mice (in which GFP expression reflected endogenous Pax7 expression quantitatively) showed that, during muscle regeneration, Pax7+ Myf5+ cells performed random DNA segregation and were myogenically committed (Rocheteau et al., 2010; Nguyen et al., 2012) that are thought inherited by amplified progeny. In 2010, Roman et al. (Roman et al., 2010), using a Pax7 knock in mouse, revealed that in this model a novel subset of Pax7+ Myf5– cells (Pax7-nGFP Lo) perform random DNA segregation and are myogenically committed (Rocheteau et al., 2010; Nguyen et al., 2012) that are thought inherited by amplified progeny. Thus, it would be interesting to investigate Pax chromatin modifications in kidney tubules and Schwann cells during responses that involve dedifferentiation of injured cells and redifferentiation of their progeny. An understanding of this Pax epigenetic switching during homeostatic tissue repair may shed light on the aberrant processes of repair that may occur in chronic kidney disease and neurofibromatosis (Pongpudpunth et al., 2010). In addition, such an understanding could be applied to the therapeutic control of PAX gain of function in ARMS and other cancers.

Conclusions

Nine Pax genes have been characterised in mammals and they are considered principal regulators of gene expression, supported by the evolutionary conservation of the paired DNA-binding domain across phyllogenies. Pax genes have important roles in the formation of the CNS, where crossregulatory feedback loops are thought to maintain and stabilise developing regions of the brain and spinal cord such that aberrant Pax gene expression perturbs neuronal fate. It is also evident that Pax genes function in gene regulatory networks in which graded gene expression outputs are the product of the robust regulatory logic of the network. Furthermore, Pax genes often function to control the balance between the proliferation and differentiation of progenitors, and a characteristic Pax ‘on/off switch’ has been described that temporally maintains a precursor in a ‘pre-differentiation’ state while poised for capacitated differentiation. The molecular control of the choice between proliferation and differentiation is thought to be a result of the use of distinct Pax DNA-binding subdomains. Developmental studies have also shown that Pax genes play a prominent role in the fate restriction, migration and differentiation of somitic and neural crest cells, where restricted downstream target gene expression primes the fate of the cell from early stages of cell identity. During emigration, however, cells are concomitantly inhibited from differentiation by Pax-mediated and environmental inhibitory effects.

Although we have focused here on the developing nervous system, the neural crest and the musculature, Pax genes regulate multiple transcriptional networks that function simultaneously to confer cellular capacity to proliferate, assume a specific cell fate and execute the differentiation program in the developing eye (Marquardt et al., 2001; Farhy et al., 2013), the pancreas (Brun and Gauthier, 2008; Gosmain et al., 2011; Hu He et al., 2011; Koopitwut et al., 2012; Pfeifer et al., 2013) and the kidney (Dressler, 2011). The prominent roles for Pax genes are linked to the perturbed expression of Pax genes seen in disease states. Importantly, miR-mediated regulation of Pax proteins (Pasut and Rudnicki, 2012; Kredo-Russo et al., 2012; Shalom-Feuerstein et al., 2012; Shaham et al., 2013) and the role of Pax proteins in the epigenetic determination of active or silenced regions of DNA is now coming to light that adds further complexity to the causes and repercussions of aberrant Pax gene expression that fuels oncogenesis and other disease.

Funding

Deposited in PMC for immediate release.

References


