

2014

# 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*

---

[10.1242/dev.091785](https://ro.ecu.edu.au/ecuworkspost2013/542)

This article was originally published as: Blake J.A., Ziman M.R. (2014). Pax genes: Regulators of lineage specification and progenitor cell maintenance. *Development (Cambridge)*, 141(4), 737-751. Original article available [here](#)

This Journal Article is posted at Research Online.

<https://ro.ecu.edu.au/ecuworkspost2013/542>

## 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*, *PAX8*, *PAX6*, *PAX3* and *PAX7*), 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*) (Lechtenberg 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 Schimmenti, 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.

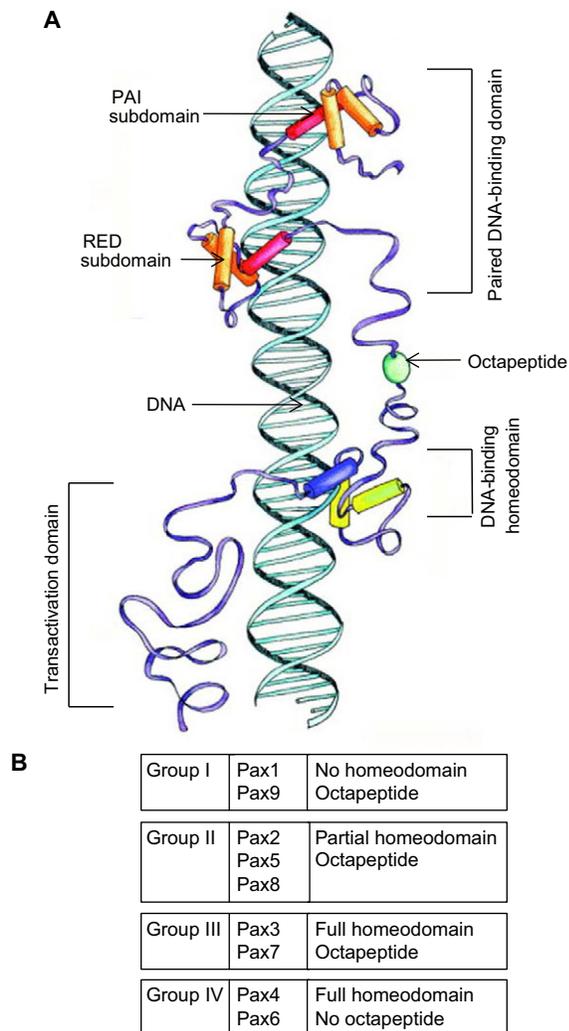
**PAX6 coordinates specification of the neuroectoderm**

*PAX6* plays an important role as early as the second week following human conception, when the formation of the CNS commences, as

School of Medical Sciences, Edith Cowan University, Joondalup, WA 6027, Australia.

\*Author for correspondence (j.blake@ecu.edu.au)

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0/>), which permits unrestricted use, distribution and reproduction in any medium provided that the original work is properly attributed.



**Fig. 1. Subclassification of PAX transcription factors according to protein structure.** (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 Tle4 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 neural 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 neural progenitor is committed, *Pax6* must be downregulated by neurogenin 2 (Neurog2) to allow differentiation (Bel-Vialar 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*

*in vivo*, it was shown that Pax6 initially regulates neocortical cell cycle progression by direct regulation of *Hmga2* (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* (hairly 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 retract 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 constituents 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/Sey</sup>* mutant mice (Götz et al., 1998); therefore, the cleavage angles of apical progenitors were assessed in both *Pax6<sup>Sey/Sey</sup>* 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 kinetochores 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**

Transcript name	Description	Function	References
<i>Pax1</i> variant 1	Encodes the longest isoform of <i>Pax1</i>		Burri et al., 1989; Schnittger et al., 1992
<i>Pax1</i> variant 2	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.		Burri et al., 1989; Smith and Tuan, 1994
<i>Pax2</i> variant a	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)	Cell differentiation during sensory placode formation (McCarroll et al., 2012); otic specification (Hans et al., 2004)	Eccles et al., 1992; Ward et al., 1994
<i>Pax2</i> variant b	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)	Maintenance of the otic placode (Mackereth et al., 2005)	Eccles et al., 1992; Ward et al., 1994
<i>Pax2</i> variant c	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.		Eccles et al., 1992; Ward et al., 1994
<i>Pax2</i> variant d	Lacks an alternate in-frame exon compared with variant e (see below). This results in an isoform that is shorter than variant e.		Tavassoli et al., 1997
<i>Pax2</i> variant e	This variant encodes the longest isoform		Eccles et al., 1992; Ward et al., 1994
<i>Pax3a</i>	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 <i>Pax3c</i> . The resulting protein lacks the paired-type homeodomain and has a shorter and distinct C terminus compared with isoform <i>Pax3c</i> .	Negative effects on melanocyte proliferation (Wang et al., 2006)	Tassabehji et al., 1992; Carezani-Gavin et al., 1992
<i>Pax3b</i>	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 <i>Pax3c</i> . The resulting protein lacks the paired-type homeodomain and has a shorter and distinct C terminus compared with isoform <i>Pax3c</i> .	Negative effects on melanocyte proliferation; reduced migration and accelerated apoptosis (Wang et al., 2006)	Tassabehji et al., 1992; Carezani-Gavin et al., 1992
<i>Pax3c</i>	The constitutive splice pattern. The resulting protein, PAX3C, is also known as PAX3.	Promotes melanocyte proliferation, migration, transformation and survival (Wang et al., 2006); required for terminal myogenic differentiation (Charytonowicz et al., 2011)	Tassabehji et al., 1992; Wang et al., 2006
<i>Pax3d</i>	Differs in the 3' UTR and contains an alternate splice pattern in the 3' coding region compared with isoform <i>Pax3c</i> . The resulting protein is longer and has a distinct C terminus compared with isoform <i>Pax3c</i> .	Promotes melanocyte proliferation, migration, transformation and survival (Wang et al., 2006); involved in undifferentiated cell maintenance and/or proliferation (Charytonowicz et al., 2011)	Barber et al., 1999; Wang et al., 2006
<i>Pax3e</i>	Differs in the 3' UTR and contains an alternate splice pattern in the 3' coding region compared with isoform <i>Pax3c</i> . The resulting protein is longer and has a distinct C terminus compared with isoform <i>Pax3c</i> .	Reduced melanocyte growth (Wang et al., 2006)	Wang et al., 2006
<i>Pax3g</i>	Differs in the 3' UTR and contains an alternate splice pattern in the 3' coding region compared with isoform <i>Pax3c</i> . The resulting protein is shorter and has a distinct C terminus compared with variant <i>Pax3c</i> .	Melanocyte migration was reduced (Wang et al., 2007)	Wang et al., 2007
<i>Pax3h</i>	Differs in the 3' UTR and contains an alternate splice pattern in the 3' coding region compared with isoform <i>Pax3c</i> . The resulting protein is shorter and has a distinct C terminus compared with isoform <i>Pax3c</i> .	Increased melanocyte proliferation, migration, survival and transformation (Wang et al., 2006)	Wang et al., 2006
<i>Pax4</i>	No data available on NCBI		
<i>Pax5</i>	No data available on NCBI		
<i>Pax6</i> variant 1	Also known as Pax6(-5a). Represents the longest transcript. Variants 1, 3, 6 and 7 encode the same isoform (a).	Neurogenesis, proliferation, regionalisation and boundary formation	Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992
<i>Pax6</i> variant 2	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.	Overexpression leads to proliferation	Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992
<i>Pax6</i> variant 3	Differs in the 5' UTR compared to variant 1. Variants 1, 3, 6 and 7 encode the same isoform (a).	Neurogenesis, proliferation, regionalisation and boundary formation	Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992
<i>Pax6</i> variant 4	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.	Overexpression leads to proliferation	Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992

**Table 1. Continued**

Transcript name	Description	Function	References
<i>Pax6</i> variant 5	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.	Overexpression leads to proliferation	Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992
<i>Pax6</i> variant 6	Differs in the 5' UTR compared with variant 1. Variants 1, 3, 6 and 7 encode the same isoform (a).	Neurogenesis, proliferation, regionalisation and boundary formation	Haubst et al., 2004; Ton et al., 1991; Glaser et al., 1992
<i>Pax6</i> variant 7	Differs in the 5' UTR compared with variant 1. Variants 1, 3, 6 and 7 encode the same isoform (a).	Neurogenesis, proliferation, regionalisation and boundary formation	Vorobyov et al., 1997; Barr et al., 1999
<i>Pax7</i> variant 1	Encodes the longest <i>Pax7</i> isoform		Vorobyov et al., 1997; Barr et al., 1999
<i>Pax7</i> variant 2	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.	A more potent transactivator of <i>Cntfr</i> (White and Ziman, 2008);	Jostes et al., 1990
<i>Pax7</i> variant 3	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.		
<i>Pax8a</i>	Encodes the longest <i>Pax8</i> isoform	Activating domain encoded by exons 10 and 11	Poleev et al., 1995
<i>Pax8b</i>	Uses an alternate splice site in the 3' coding region compared with isoform <i>Pax8a</i> that results in a frameshift and an early stop codon. It encodes an isoform that has a shorter and distinct C terminus compared to <i>Pax8a</i> .	Antagonistic role to the activating domain	Poleev et al., 1995
<i>Pax8d</i>	Lacks two alternate exons compared with isoform <i>Pax8a</i> that results in a frameshift and an early stop codon. The encoded isoform is shorter and has a distinct C terminus compared with <i>Pax8a</i> .		Poleev et al., 1995
<i>Pax8e</i>	Lacks three alternate exons compared to isoform <i>Pax8a</i> that results in a frameshift and an early stop codon. The encoded isoform is shorter and has a distinct C terminus compared with <i>Pax8a</i> .		
<i>Pax9</i>	No data available on NCBI		

*Cntfr*, 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).

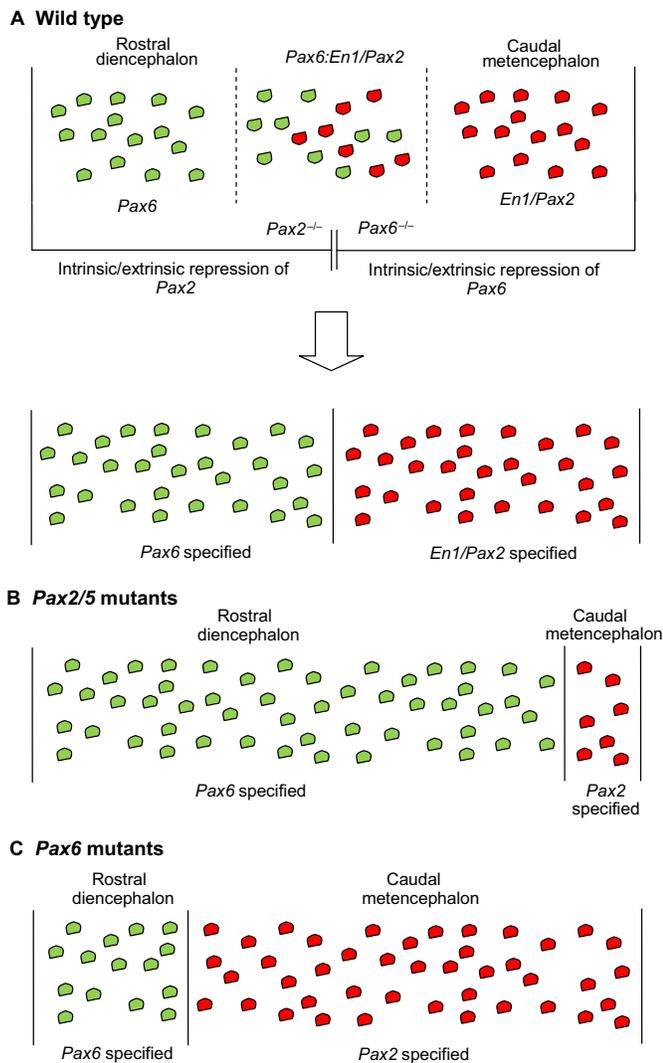
Although the structures of their DNA-binding domains are similar (Vorobyov et al., 1997), differences in *Pax3* and *Pax7* function and regulation during NC development have been identified. Using *Pax7*-cre/reporter mice, *Pax7*-expressing precursors were noted to be distinct from *Pax3*-expressing NC precursors, and principally contribute to the cranial NC derivatives of the frontal and 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* (hairly and enhancer of split 1; which is involved in neural stem cell maintenance), *Neurog2* (which is involved in NC neurogenesis) and *Tgfb2* (transforming growth factor  $\beta$ 2) (which in turn represses *Pax3* promoter activity on *Hes1* and *Neurog2*) (Therault et al., 2005; Mayanil et al., 2006; Nakazaki et al., 2008; Medic and Ziman, 2010). Using mice bred for a double heterozygous *Pax3* and *Tgfb2* phenotype, Nakazaki et al. (Nakazaki et al., 2009) demonstrated that the *Tgfb2*<sup>-/-</sup> *Pax3*<sup>+/-</sup> phenotype of open neural tube and bifid spine was reversed in 85% of animals following the loss of one *Pax3* allele (*Tgfb2*<sup>-/-</sup> *Pax3*<sup>+/-</sup>). Results were attributed to the opposing roles of *PAX3* and *TGF $\beta$ 2* 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*, *Tgfb2*, *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 *Tgfb2* 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**

Name/species	Mutation	Pathology	References
'Undulated'/mouse	G15S (paired-domain) <i>Pax1</i>	Abnormalities in the sternum and parts of the vertebral column in heterozygotes	Balling et al., 1988
'Scoliosis'/mouse	2.0 kb and 4.5 kb deletions exons 1 to 4, respectively, in <i>Pax1</i>	Homozygous mice show lumbar scoliosis and a kinked tail	Adham et al., 2005
Papillorenal syndrome/human	Heterogeneous mutations to <i>PAX2</i>	Ocular and renal anomalies, vesicoureteral reflux, high frequency hearing loss, central nervous system anomalies and/or genital anomalies	Schimmenti et al., 1997; Eccles and Schimmenti, 1999; Tellier et al., 1998; Devriendt et al., 1998; Amiel et al., 2000; Nishimoto et al., 2001; Higashide et al., 2005; Bower et al., 2012
Craniofacial-deafness-hand syndrome/human	Asn47-to-lys; asn47-to-his (paired domain) <i>PAX3</i>	Flat facial profile, hypertelorism, hypoplastic nose with slitlike nares, sensorineural hearing loss. small maxilla, absent or small nasal bones and ulnar deviation of the hands	Hoth et al., 1993; Asher et al., 1996
Rhabdomyosarcoma 2, alveolar/human	Translocation of the <i>PAX3</i> gene on chromosome 2 or <i>PAX7</i> gene on chromosome 1 with the <i>FKHR/FOXO1A</i> gene on chromosome 13	Childhood sarcoma	Douglass et al., 1987; Wang-Wuu et al., 1988; Hayashi et al., 1988; Barr et al., 1991; Barr et al., 1993; Fredericks et al., 1995
Waardenburg syndrome, type 1/human	Heterogeneous mutations in <i>PAX3</i>	Pigmentary abnormalities of the hair, skin and eyes; congenital sensorineural hearing loss; dystopia canthorum; lateral displacement of the ocular inner canthi	Tassabehji et al., 1992; Baldwin et al., 1992; Ishikiriyama, 1993; Baldwin et al., 1995
Waardenburg syndrome, type 3/human	Heterogeneous mutations in <i>PAX3</i>	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	Hoth et al., 1993; Zlotogora, 1995; Tekin et al., 2001; Wollnik et al., 2003
'Spotch'/mouse	A-to-T transversion of the AG splice acceptor of intron 3 of <i>Pax3</i>	Neural tube, neural crest, severe limb and trunk muscle defects	Epstein et al., 1991
'Spotch-delayed'/mouse	G9R (paired domain) <i>Pax3</i>	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	Yang and Trasler, 1991
Ketosis-prone diabetes mellitus/human	Ethnic-specific gene variants in <i>PAX4</i>	Severe hyperglycemia and ketosis	Sobngwi and Gautier, 2002; Mauvais-Jarvis et al., 2004
Diabetes mellitus, type 2/human	Heterogenous mutations of <i>PAX4</i>	Weight loss, fatigue, polydipsia, polyuria and hyperglycemia	Shimajiri et al., 2001
Lymphoplasmacytoid lymphoma/human	Haploinsufficiency of <i>PAX5</i>	Leukaemia	Nutt and Busslinger, 1999; Rolink et al., 1999; Mullighan et al., 2007
Small eye/mouse	Mutation in <i>Pax6</i>	Aniridia and corneal abnormalities	Glaser et al., 1990; Hill et al., 1991; Matsuo et al., 1993; Ramaesh et al., 2003; Ramaesh et al., 2006
Aniridia/human	Mutation in <i>PAX6</i>	Noticeable iris hypoplasia	Shaw et al., 1960; Elsas et al., 1977; Glaser et al., 1994; Sisodiya et al., 2001
Coloboma of optic nerve/human	Mutation in <i>PAX6</i>	Detachment of the macula	Jonas and Freisler, 1997; Hornby et al., 2000; Azuma et al., 2003
Foveal hyperplasia/human	Mutation in <i>PAX6</i>	Subnormal visual acuity and congenital nystagmus	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
Gillespie syndrome/human	Mutation in <i>PAX6</i>	Aniridia, cerebellar ataxia and mental retardation	Gillespie, 1965; Sarsfield, 1971; Crawford et al., 1979; Lechtenberg and Ferretti, 1981; Wittig 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
Keratitis/human	Mutation in <i>PAX6</i>	Childhood corneal clouding	Hanson et al., 1994; Yang et al., 2004
Optic nerve hypoplasia/human	Mutation in <i>PAX6</i>	Bilateral optic nerve hypoplasia, poor visual acuity and wandering movements of the eyes	Hanson et al., 1994; Yang et al., 2004
Peters anomaly/human	Mutation in <i>PAX6</i>	Central corneal leukoma, absence of the posterior corneal stroma and lenticular attachments to the central aspect of the posterior cornea	Hanson et al., 1994; Yang et al., 2004
Hypothyroidism, congenital, due to thyroid dysgenesis or hypoplasia/human	Heterogenous mutations of <i>PAX8</i>	Congenital hypothyroidism, ectopic thyroid gland, athyreotic cretinism	Macchia et al., 1998; Vilain et al., 2001; Congdon et al., 2001; Meeus et al., 2004
Tooth agenesis, selective, 3/human	Heterogenous mutations of <i>PAX9</i>	Oligodontia and hypodontia	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
'Pax9-neo'/mouse	Hypomorphic <i>Pax9</i> allele	Oligodontia	Kist et al., 2005



**Fig. 2. Regional specification of brain neuroblasts.** (A) In the early brain, *Pax6* (green) is strongly induced in the rostral region as *Pax2* (red) is induced in the caudal region, giving rise to an overlap in *Pax6* and *Pax2* expression at the di-mesencephalic boundary. In this region of overlapping expression, *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. (B) In *Pax2<sup>-/-</sup> Pax5<sup>-/-</sup>* mice, the mesencephalon primordium is lost due to expanded expression of *Pax6* (Schwarz et al., 1999). (C) Altered regionalisation, such as that occurring in *Pax6* *Sey* mutant mice (which harbour a mutation in the donor splice site for exon 10 of *Pax6*), leads to a misexpression of genes that are characteristic of the neighbouring brain region and a change in neuronal fate (Mastick et al., 1997).

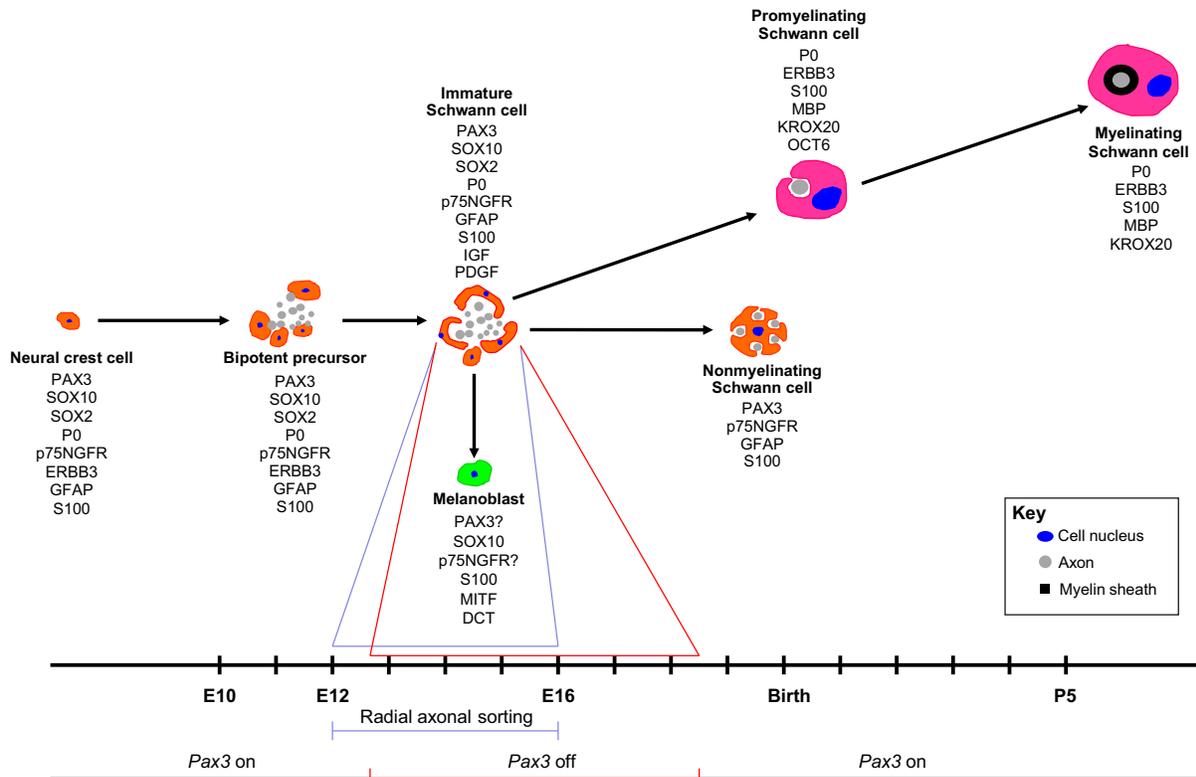
uncertainty about *Pax3* requirement for NC migration (Li et al., 1999; Conway et al., 2000; Kwang et al., 2002; Chan et al., 2004) has been addressed recently by looking at the role of *Pax3* in migratory cardiac NC cells using the *Pax3* mutant *Splotch* mouse models, all six of which have different 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 progenitor 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  $\beta$ -neuregulin 1-secreting axon tracts, their maintenance is regulated by *Sox2*, *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*



**Fig. 3. Development of Schwann cells and melanocytes from neural crest cells.** Melanoblasts diverge from bi-potent 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;

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 ISCs 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 (Nitzan 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 (Nitzan 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 (Christ 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 (Nitzan and Kalcheim, 2013). During this somitic patterning phase, Pax3 is expressed in the presomitic 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

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<sup>+</sup> 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 delamination 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 perpendicular planar shift of cell division occurs in the central sheet concurrent with generation of dermal and myoblast progenitors; here, *Pax7*<sup>+</sup> myoblast progenitors undergo asymmetric division and delamination results in the production of a basal Pax7<sup>+</sup> 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<sup>+</sup> Pax7<sup>+</sup> 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<sup>+</sup> Pax7<sup>+</sup> offspring either enter the myogenic program through upregulation of the myogenic regulatory factors *Myf5* and *Myod1* or retain a Pax3<sup>+</sup> Pax7<sup>+</sup> *Mrf*<sup>-</sup> (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 (Günther 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 *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

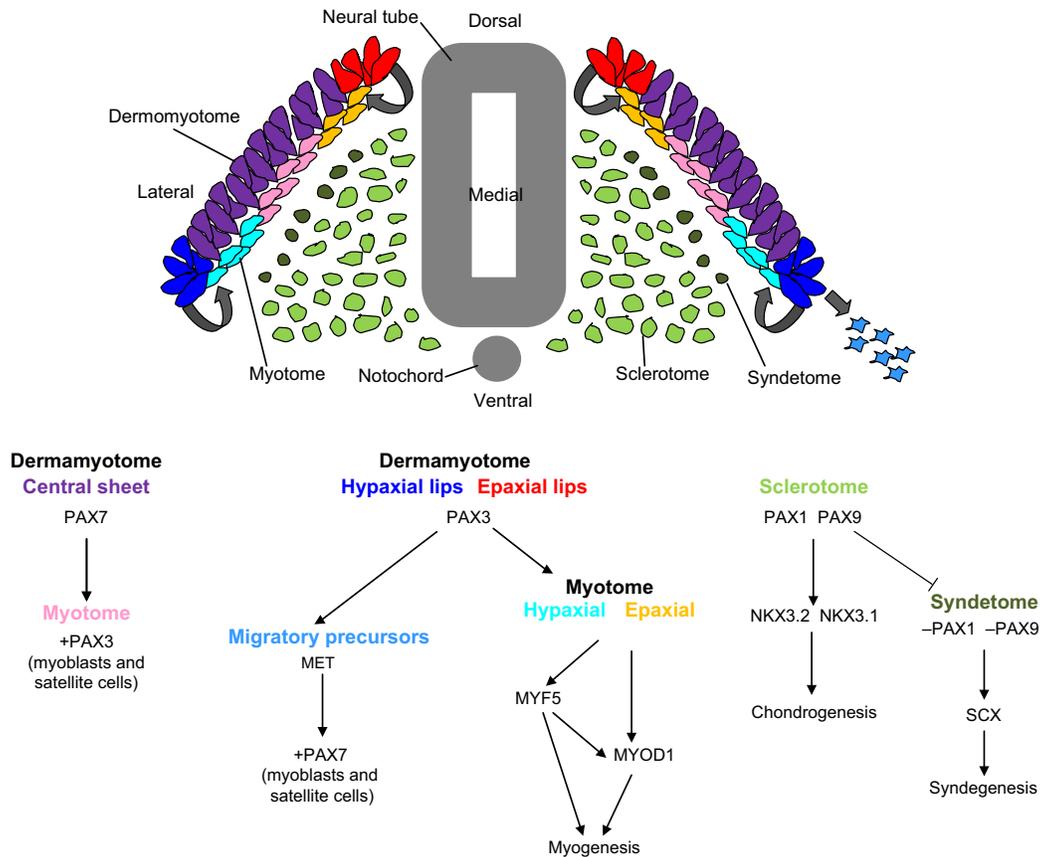
The important roles of Pax genes in development underscores their function in adult tissue regeneration and the repercussions of their aberrant loss, overexpression, re-expression or persistent expression in association with pathology (Table 2) and, in particular, cancer (Li and Eccles, 2012). The Pax 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 PAX3: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 multinucleated 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



**Fig. 4. Pax gene hierarchies involved in the development of the progenitor populations of skeletal muscle, cartilage and tendon.** Cells in the ventromedial part of the somite de-epithelialise and form the sclerotome (light green), in which *Pax1* and *Pax9* are expressed in a *Shh*-dependent manner. As the dermomyotome elongates dorsomedially and ventrolaterally, *PAX3* becomes restricted to the epaxial (red) and hypaxial (dark blue) lips, where a secondary generation of myocytes delaminate and migrate to form the epaxial (orange) and hypaxial (light blue) myotome. In the limb region, *PAX3*<sup>+</sup> progenitors delaminate from the hypaxial dermomyotome (light blue) and migrate into the developing limbs to provide a pool of progenitors. In these cells, *PAX3* regulates the hepatocyte growth factor receptor *Met*, which is necessary for hypaxial delamination and migration. *Pax7* is expressed principally within the central sheet of the dermomyotome (purple); when the final somite dissociates, *Pax7*<sup>+</sup> progenitors delaminate into the developing myotome. These myotomal *Pax3*<sup>+</sup> *Pax7*<sup>+</sup> cells (pink) retain a progenitor state in order to become a resident population necessary for skeletal muscle growth as development proceeds. The syndetome (dark green) originates from the dorsolateral edge of the sclerotome, as *Pax1* and *Pax9* are downregulated and scleraxis (*Scx*) upregulation leads to syndegeneration. Pax, paired homeobox; MYOD1, myogenic differentiation antigen 1; MYF5, myogenic factor 5; NKX, NK homeobox; SCX, scleraxis.

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 post-mitotic 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 reinstatement 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<sup>+</sup> Myf5<sup>-</sup>* cells through basal-apical asymmetric cell divisions. Isolation and transplantation of these cells into muscle revealed that they underwent differentiation while *Pax7<sup>+</sup> Myf5<sup>-</sup>* 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-nGFP<sup>Hi</sup>* cells retain template DNA strands and expressed stem cell markers while *Pax7-nGFP<sup>Lo</sup>* cells performed random DNA segregation and were myogenically committed (Rocheteau et al., 2012). Taken together, these studies support a role for *Pax7* in the acquisition of a stem cell fate, which is characterised by high levels of PAX7 and the lack of myogenic fate marker expression such as *Myf5*. The means by which this inheritance is designated is associated with the genomic and/or epigenomic regulation of stemness in stem cells (Kawabe et al., 2012) and looks to be an intriguing area of future research in the cancer stem cell field.

#### 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 phylogenies. 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

- Acampora, D., Mazan, S., Lallemand, Y., Avantaggiato, V., Maury, M., Simeone, A. and Brûlet, P. (1995). Forebrain and midbrain regions are deleted in *Otx2<sup>-/-</sup>* mutants due to a defective anterior neuroectoderm specification during gastrulation. *Development* **121**, 3279-3290.
- Adameyko, I. and Lallemand, F. (2010). Glial versus melanocyte cell fate choice: Schwann cell precursors as a cellular origin of melanocytes. *Cell. Mol. Life Sci.* **67**, 3037-3055.
- Adameyko, I., Lallemand, F., Aquino, J. B., Pereira, J. A., Topilko, P., Müller, T., Fritz, N., Beljajeva, A., Mochii, M., Liste, I. et al. (2009). Schwann cell precursors from nerve innervation are a cellular origin of melanocytes in skin. *Cell* **139**, 366-379.
- Adham, I. M., Gille, M., Gamel, A. J., Reis, A., Dressel, R., Steding, G., Brand-Saberi, B. and Engel, W. (2005). The scoliosis (*sco*) mouse: a new allele of *Pax1*. *Cytogenet. Genome Res.* **111**, 16-26.
- Agoston, Z. and Schulte, D. (2009). *Meis2* competes with the Groucho co-repressor *Tle4* for binding to *Otx2* and specifies tectal fate without induction of a secondary midbrain-hindbrain boundary organizer. *Development* **136**, 3311-3322.
- Agoston, Z., Li, N., Haslinger, A., Wizenmann, A. and Schulte, D. (2012). Genetic and physical interaction of *Meis2*, *Pax3* and *Pax7* during dorsal midbrain development. *BMC Dev. Biol.* **12**, 10.
- Amiel, J., Audollent, S., Joly, D., Dureau, P., Salomon, R., Tellier, A. L., Augé, J., Bouissou, F., Antignac, C., Gubler, M. C. et al. (2000). PAX2 mutations in renal-coloboma syndrome: mutational hotspot and germline mosaicism. *Eur. J. Hum. Genet.* **8**, 820-826.
- Asami, M., Pilz, G. A., Ninkovic, J., Godinho, L., Schroeder, T., Huttner, W. B. and Götz, M. (2011). The role of *Pax6* in regulating the orientation and mode of cell division of progenitors in the mouse cerebral cortex. *Development* **138**, 5067-5078.
- Asher, J. H., Jr, Sommer, A., Morell, R. and Friedman, T. B. (1996). Missense mutation in the paired domain of PAX3 causes craniofacial-deafness-hand syndrome. *Hum. Mutat.* **7**, 30-35.
- Azuma, N., Nishina, S., Yanagisawa, H., Okuyama, T. and Yamada, M. (1996). PAX6 missense mutation in isolated foveal hypoplasia. *Nat. Genet.* **13**, 141-142.

- Azuma, N., Yamaguchi, Y., Handa, H., Tadokoro, K., Asaka, A., Kawase, E. and Yamada, M. (2003). Mutations of the PAX6 gene detected in patients with a variety of optic-nerve malformations. *Am. J. Hum. Genet.* **72**, 1565-1570.
- Bajard, L., Relaix, F., Lagna, M., Rocancourt, D., Daubas, P. and Buckingham, M. E. (2006). A novel genetic hierarchy functions during hypaxial myogenesis: Pax3 directly activates Myf5 in muscle progenitor cells in the limb. *Genes Dev.* **20**, 2450-2464.
- Balaskas, N., Ribeiro, A., Panovska, J., Dessaud, E., Sasai, N., Page, K. M., Briscoe, J. and Ribes, V. (2012). Gene regulatory logic for reading the Sonic Hedgehog signaling gradient in the vertebrate neural tube. *Cell* **148**, 273-284.
- Baldwin, C. T., Hoth, C. F., Amos, J. A., da-Silva, E. O. and Milunsky, A. (1992). An exonic mutation in the HuP2 paired domain gene causes Waardenburg's syndrome. *Nature* **355**, 637-638.
- Baldwin, C. T., Hoth, C. F., Macina, R. A. and Milunsky, A. (1995). Mutations in PAX3 that cause Waardenburg syndrome type I: ten new mutations and review of the literature. *Am. J. Med. Genet.* **58**, 115-122.
- Balling, R., Deutsch, U. and Gruss, P. (1988). undulated, a mutation affecting the development of the mouse skeleton, has a point mutation in the paired box of Pax 1. *Cell* **55**, 531-535.
- Barber, T. D., Barber, M. C., Cloutier, T. E. and Friedman, T. B. (1999). PAX3 gene structure, alternative splicing and evolution. *Gene* **237**, 311-319.
- Barr, F. G., Sellinger, B. and Emanuel, B. S. (1991). Localization of the rhabdomyosarcoma t(2;13) breakpoint on a physical map of chromosome 13. *Genomics* **11**, 941-947.
- Barr, F. G., Galili, N., Holick, J., Biegel, J. A., Rovera, G. and Emanuel, B. S. (1993). Rearrangement of the PAX3 paired box gene in the paediatric solid tumour alveolar rhabdomyosarcoma. *Nat. Genet.* **3**, 113-117.
- Barr, F. G., Nauta, L. E., Davis, R. J., Schäfer, B. W., Nycum, L. M. and Biegel, J. A. (1996). In vivo amplification of the PAX3-FKHR and PAX7-FKHR fusion genes in alveolar rhabdomyosarcoma. *Hum. Mol. Genet.* **5**, 15-21.
- Barr, F. G., Fitzgerald, J. C., Ginsberg, J. P., Vanella, M. L., Davis, R. J. and Bennicelli, J. L. (1999). Predominant expression of alternative PAX3 and PAX7 forms in myogenic and neural tumor cell lines. *Cancer Res.* **59**, 5443-5448.
- Basch, M. L. and Bronner-Fraser, M. (2006). Neural crest inducing signals. *Adv. Exp. Med. Biol.* **589**, 24-31.
- Basch, M. L., Bronner-Fraser, M. and Garcia-Castro, M. I. (2006). Specification of the neural crest occurs during gastrulation and requires Pax7. *Nature* **441**, 218-222.
- Bel-Vialar, S., Medevielle, F. and Pituello, F. (2007). The on/off of Pax6 controls the tempo of neuronal differentiation in the developing spinal cord. *Dev. Biol.* **305**, 659-673.
- Ben-Yair, R., Kahane, N. and Kalcheim, C. (2011). LGN-dependent orientation of cell divisions in the dermomyotome controls lineage segregation into muscle and dermis. *Development* **138**, 4155-4166.
- Bennicelli, J. L., Edwards, R. H. and Barr, F. G. (1996). Mechanism for transcriptional gain of function resulting from chromosomal translocation in alveolar rhabdomyosarcoma. *Proc. Natl. Acad. Sci. USA* **93**, 5455-5459.
- Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K. et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315-326.
- Blanchard, A. D., Sinanan, A., Parmantier, E., Zwart, R., Broos, L., Meijer, D., Meier, C., Jessen, K. R. and Mirsky, R. (1996). Oct-6 (SCIP/Tst-1) is expressed in Schwann cell precursors, embryonic Schwann cells, and postnatal myelinating Schwann cells: comparison with Oct-1, Krox-20, and Pax-3. *J. Neurosci. Res.* **46**, 630-640.
- Bower, M., Salomon, R., Allanson, J., Antignac, C., Benedicenti, F., Benetti, E., Binenbaum, G., Jensen, U. B., Cochot, P., DeCramer, S. et al. (2012). Update of PAX2 mutations in renal coloboma syndrome and establishment of a locus-specific database. *Hum. Mutat.* **33**, 457-466.
- Brand-Saberi, B. and Christ, B. (2000). Evolution and development of distinct cell lineages derived from somites. *Curr. Top. Dev. Biol.* **48**, 1-42.
- Brent, A. E., Schweitzer, R. and Tabin, C. J. (2003). A somitic compartment of tendon progenitors. *Cell* **113**, 235-248.
- Britsch, S., Goerich, D. E., Riethmacher, D., Peirano, R. I., Rossner, M., Nave, K. A., Birchmeier, C. and Wegner, M. (2001). The transcription factor Sox10 is a key regulator of peripheral glial development. *Genes Dev.* **15**, 66-78.
- Bronner, M. E. (2012). A career at the interface of cell and developmental biology: a view from the crest. *Mol. Biol. Cell* **23**, 4151-4153.
- Brun, T. and Gauthier, B. R. (2008). A focus on the role of Pax4 in mature pancreatic islet beta-cell expansion and survival in health and disease. *J. Mol. Endocrinol.* **40**, 37-45.
- Buckingham, M. and Relaix, F. (2007). The role of Pax genes in the development of tissues and organs: Pax3 and Pax7 regulate muscle progenitor cell functions. *Annu. Rev. Cell Dev. Biol.* **23**, 645-673.
- Buckingham, M., Bajard, L., Chang, T., Daubas, P., Hadchouel, J., Meilhac, S., Montarras, D., Rocancourt, D. and Relaix, F. (2003). The formation of skeletal muscle: from somite to limb. *J. Anat.* **202**, 59-68.
- Burri, M., Tromvoukis, Y., Bopp, D., Frigerio, G. and Noll, M. (1989). Conservation of the paired domain in metazoans and its structure in three isolated human genes. *EMBO J.* **8**, 1183-1190.
- Bylund, M., Andersson, E., Novitsch, B. G. and Muhr, J. (2003). Vertebrate neurogenesis is counteracted by Sox1-3 activity. *Nat. Neurosci.* **6**, 1162-1168.
- Carezzani-Gavin, M., Clarren, S. K. and Steege, T. (1992). Waardenburg syndrome associated with meningomyelocele. *Am. J. Med. Genet.* **42**, 135-136.
- Chan, W. Y., Cheung, C. S., Yung, K. M. and Copp, A. J. (2004). Cardiac neural crest of the mouse embryo: axial level of origin, migratory pathway and cell autonomy of the splotch (Sp2H) mutant effect. *Development* **131**, 3367-3379.
- Charytonowicz, E., Cordon-Cardo, C., Matushansky, I. and Ziman, M. (2009). Alveolar rhabdomyosarcoma: is the cell of origin a mesenchymal stem cell? *Cancer Lett.* **279**, 126-136.
- Charytonowicz, E., Matushansky, I., Castillo-Martin, M., Hricik, T., Cordon-Cardo, C. and Ziman, M. (2011). Alternate PAX3 and PAX7 C-terminal isoforms in myogenic differentiation and sarcomagenesis. *Clin. Transl. Oncol.* **13**, 194-203.
- Chi, N. and Epstein, J. A. (2002). Getting your Pax straight: Pax proteins in development and disease. *Trends Genet.* **18**, 41-47.
- Cho, E. A., Prindle, M. J. and Dressler, G. R. (2003). BRCT domain-containing protein PTIP is essential for progression through mitosis. *Mol. Cell. Biol.* **23**, 1666-1673.
- Christ, B., Huang, R. and Wiltig, J. (2000). The development of the avian vertebral column. *Anat. Embryol. (Berl.)* **202**, 179-194.
- Congdon, T., Nguyen, L. Q., Nogueira, C. R., Habiby, R. L., Medeiros-Neto, G. and Kopp, P. (2001). A novel mutation (Q40P) in PAX8 associated with congenital hypothyroidism and thyroid hypoplasia: evidence for phenotypic variability in mother and child. *J. Clin. Endocrinol. Metab.* **86**, 3962-3967.
- Conway, S. J., Henderson, D. J., Kirby, M. L., Anderson, R. H. and Copp, A. J. (1997). Development of a lethal congenital heart defect in the splotch (Pax3) mutant mouse. *Cardiovasc. Res.* **36**, 163-173.
- Conway, S. J., Bundy, J., Chen, J., Dickman, E., Rogers, R. and Will, B. M. (2000). Decreased neural crest stem cell expansion is responsible for the conotruncal heart defects within the splotch (Sp(2H))/Pax3 mouse mutant. *Cardiovasc. Res.* **47**, 314-328.
- Cramer, S. F. and Fesyuk, A. (2012). On the development of neurocutaneous units—implications for the histogenesis of congenital, acquired, and dysplastic nevi. *Am. J. Dermatopathol.* **34**, 60-81.
- Crawford, M. D., Harcourt, R. B. and Shaw, P. A. (1979). Non-progressive cerebellar ataxia, aplasia of pupillary zone of iris, and mental subnormality (Gillespie's syndrome) affecting 3 members of a non-consanguineous family in 2 generations. *J. Med. Genet.* **16**, 373-378.
- Curran, R. E. and Robb, R. M. (1976). Isolated foveal hypoplasia. *Arch. Ophthalmol.* **94**, 48-50.
- Das, P., Stockton, D. W., Bauer, C., Shaffer, L. G., D'Souza, R. N., Wright, T. and Patel, P. I. (2002). Haploinsufficiency of PAX9 is associated with autosomal dominant hypodontia. *Hum. Genet.* **110**, 371-376.
- Devriendt, K., Matthijs, G., Van Damme, B., Van Caesbroeck, D., Eccles, M., Vanreterghem, Y., Fryns, J. P. and Leys, A. (1998). Missense mutation and hexanucleotide duplication in the PAX2 gene in two unrelated families with renal-coloboma syndrome (MIM 120330). *Hum. Genet.* **103**, 149-153.
- Diao, Y., Guo, X., Li, Y., Sun, K., Lu, L., Jiang, L., Fu, X., Zhu, H., Sun, H., Wang, H. et al. (2012). Pax3/7BP is a Pax7- and Pax3-binding protein that regulates the proliferation of muscle precursor cells by an epigenetic mechanism. *Cell Stem Cell* **11**, 231-241.
- Dietrich, S. (1999). Regulation of hypaxial muscle development. *Cell Tissue Res.* **296**, 175-182.
- Doddrell, R. D., Dun, X. P., Moate, R. M., Jessen, K. R., Mirsky, R. and Parkinson, D. B. (2012). Regulation of Schwann cell differentiation and proliferation by the Pax-3 transcription factor. *Glia* **60**, 1269-1278.
- Douglass, E. C., Valentine, M., Etcubanas, E., Parham, D., Webber, B. L., Houghton, P. J., Houghton, J. A. and Green, A. A. (1987). A specific chromosomal abnormality in rhabdomyosarcoma. *Cytogenet. Cell Genet.* **45**, 148-155.
- Dressler, G. R. (2011). Patterning and early cell lineage decisions in the developing kidney: the role of Pax genes. *Pediatr. Nephrol.* **26**, 1387-1394.
- Dziarnaga, A., Clark, P., Stayner, C., Julien, J. P., Torban, E., Goodyer, P. and Eccles, M. (2003). Ureteric bud apoptosis and renal hypoplasia in transgenic PAX2-Bax fetal mice mimics the renal-coloboma syndrome. *J. Am. Soc. Nephrol.* **14**, 2767-2774.
- Eberhard, D., Jiménez, G., Heavey, B. and Busslinger, M. (2000). Transcriptional repression by Pax5 (BSAP) through interaction with corepressors of the Groucho family. *EMBO J.* **19**, 2292-2303.
- Eccles, M. R. and Schimmenti, L. A. (1999). Renal-coloboma syndrome: a multi-system developmental disorder caused by PAX2 mutations. *Clin. Genet.* **56**, 1-9.
- Eccles, M. R., Wallis, L. J., Fidler, A. E., Spurr, N. K., Goodfellow, P. J. and Reeve, A. E. (1992). Expression of the PAX2 gene in human fetal kidney and Wilms' tumor. *Cell Growth Differ.* **3**, 279-289.
- Elsas, F. J., Maumenee, I. H., Kenyon, K. R. and Yoder, F. (1977). Familial aniridia with preserved ocular function. *Am. J. Ophthalmol.* **83**, 718-724.
- Engleka, K. A., Gitler, A. D., Zhang, M., Zhou, D. D., High, F. A. and Epstein, J. A. (2005). Insertion of Cre into the Pax3 locus creates a new allele of Splotch and identifies unexpected Pax3 derivatives. *Dev. Biol.* **280**, 396-406.
- Epstein, J. A. (1996). Pax3, neural crest and cardiovascular development. *Trends Cardiovasc. Med.* **6**, 255-260.
- Epstein, D. J., Vekemans, M. and Gros, P. (1991). Splotch (Sp2H), a mutation affecting development of the mouse neural tube, shows a deletion within the paired homeodomain of Pax-3. *Cell* **67**, 767-774.
- Ericson, J., Rashbass, P., Schedl, A., Brenner-Morton, S., Kawakami, A., van Heyningen, V., Jessell, T. M. and Briscoe, J. (1997). Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. *Cell* **90**, 169-180.
- Ernoffs, P. (2010). Cellular origin and developmental mechanisms during the formation of skin melanocytes. *Exp. Cell Res.* **316**, 1397-1407.

- Fang, M., Ren, H., Liu, J., Cadigan, K. M., Patel, S. R. and Dressler, G. R. (2009). Drosophila *ptip* is essential for anterior/posterior patterning in development and interacts with the PcG and trxG pathways. *Development* **136**, 1929-1938.
- Farhy, C., Elgart, M., Shapira, Z., Oron-Karni, V., Yaron, O., Menuchin, Y., Rechavi, G. and Ashery-Padan, R. (2013). Pax6 is required for normal cell-cycle exit and the differentiation kinetics of retinal progenitor cells. *PLoS ONE* **8**, e76489.
- François, J., Lentini, F. and de Rouck, F. (1984). Gillespie's syndrome (incomplete aniridia, cerebellar ataxia and oligophrenia). *Ophthalmic Paediatr. Genet.* **4**, 29-32.
- Franz, T. (1990). Defective ensheathment of motoric nerves in the Splotch mutant mouse. *Acta Anat. (Basel)* **138**, 246-253.
- Frazier-Bowers, S. A., Scott, M. R., Cavender, A., Mensah, J. and D'Souza, R. N. (2002). Mutational analysis of families affected with molar oligodontia. *Connect. Tissue Res.* **43**, 296-300.
- Fredericks, W. J., Galili, N., Mukhopadhyay, S., Rovera, G., Bennicelli, J., Barr, F. G. and Rauscher, F. J., 3rd (1995). The PAX3-FKHR fusion protein created by the t(2;13) translocation in alveolar rhabdomyosarcomas is a more potent transcriptional activator than PAX3. *Mol. Cell. Biol.* **15**, 1522-1535.
- Galli, L. M., Knight, S. R., Barnes, T. L., Doak, A. K., Kadzik, R. S. and Burrus, L. W. (2008). Identification and characterization of subpopulations of Pax3 and Pax7 expressing cells in developing chick somites and limb buds. *Dev. Dyn.* **237**, 1862-1874.
- Gillespie, F. D. (1965). Aniridia, Cerebellar Ataxia, and Oligophrenia in Siblings. *Arch. Ophthalmol.* **73**, 338-341.
- Glaser, T., Lane, J. and Housman, D. (1990). A mouse model of the aniridia-Wilms tumor deletion syndrome. *Science* **250**, 823-827.
- Glaser, T., Walton, D. S. and Maas, R. L. (1992). Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. *Nat. Genet.* **2**, 232-239.
- Glaser, T., Ton, C. C., Mueller, R., Petzl-Erler, M. L., Oliver, C., Nevin, N. C., Housman, D. E. and Maas, R. L. (1994). Absence of PAX6 gene mutations in Gillespie syndrome (partial aniridia, cerebellar ataxia, and mental retardation). *Genomics* **19**, 145-148.
- Gosmain, Y., Cheyssac, C., Heddad Masson, M., Dibner, C. and Philippe, J. (2011). Glucagon gene expression in the endocrine pancreas: the role of the transcription factor Pax6 in  $\alpha$ -cell differentiation, glucagon biosynthesis and secretion. *Diabetes Obes. Metab.* **13 Suppl.** **1**, 31-38.
- Götz, M., Stoykova, A. and Gruss, P. (1998). Pax6 controls radial glia differentiation in the cerebral cortex. *Neuron* **21**, 1031-1044.
- Goulding, M. D., Chalepakis, G., Deutsch, U., Erselius, J. R. and Gruss, P. (1991). Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J.* **10**, 1135-1147.
- Goulding, M., Sterrer, S., Fleming, J., Balling, R., Nadeau, J., Moore, K. J., Brown, S. D., Steel, K. P. and Gruss, P. (1993). Analysis of the Pax-3 gene in the mouse mutant splotch. *Genomics* **17**, 355-363.
- Graziano, C., D'Elia, A. V., Mazzanti, L., Moscano, F., Guidelli Guidi, S., Scarano, E., Turchetti, D., Franzoni, E., Romeo, G., Damante, G. et al. (2007). A de novo nonsense mutation of PAX6 gene in a patient with aniridia, ataxia, and mental retardation. *Am. J. Med. Genet. A.* **143A**, 1802-1805.
- Günther, S., Kim, J., Kostin, S., Lepper, C., Fan, C. M. and Braun, T. (2013). Myf5-positive satellite cells contribute to Pax7-dependent long-term maintenance of adult muscle stem cells. *Cell Stem Cell* **13**, 590-601.
- Hackenbruch, Y., Meerhoff, E., Besio, R. and Cardoso, H. (1975). Familial bilateral optic nerve hypoplasia. *Am. J. Ophthalmol.* **79**, 314-320.
- Hans, S., Liu, D. and Westerfield, M. (2004). Pax8 and Pax2a function synergistically in otic specification, downstream of the Foxi1 and Dlx3b transcription factors. *Development* **131**, 5091-5102.
- Hanson, I. M., Fletcher, J. M., Jordan, T., Brown, A., Taylor, D., Adams, R. J., Punnett, H. H. and van Heyningen, V. (1994). Mutations at the PAX6 locus are found in heterogeneous anterior segment malformations including Peters' anomaly. *Nat. Genet.* **6**, 168-173.
- Hanson, I., Churchill, A., Love, J., Axton, R., Moore, T., Clarke, M., Meire, F. and van Heyningen, V. (1999). Missense mutations in the most ancient residues of the PAX6 paired domain underlie a spectrum of human congenital eye malformations. *Hum. Mol. Genet.* **8**, 165-172.
- Haubst, N., Berger, J., Radjendirane, V., Graw, J., Favor, J., Saunders, G. F., Stoykova, A. and Götz, M. (2004). Molecular dissection of Pax6 function: the specific roles of the paired domain and homeodomain in brain development. *Development* **131**, 6131-6140.
- Hayashi, Y., Inaba, T., Hanada, R. and Yamamoto, K. (1988). Translocation 2;8 in a congenital rhabdomyosarcoma. *Cancer Genet. Cytogenet.* **30**, 343-345.
- Henion, P. D. and Weston, J. A. (1997). Timing and pattern of cell fate restrictions in the neural crest lineage. *Development* **124**, 4351-4359.
- Higashide, T., Wada, T., Sakurai, M., Yokoyama, H. and Sugiyama, K. (2005). Macular abnormalities and optic disk anomaly associated with a new PAX2 missense mutation. *Am. J. Ophthalmol.* **139**, 203-205.
- Hill, R. E., Favor, J., Hogan, B. L., Ton, C. C., Saunders, G. F., Hanson, I. M., Prosser, J., Jordan, T., Hastie, N. D. and van Heyningen, V. (1991). Mouse small eye results from mutations in a paired-like homeobox-containing gene. *Nature* **354**, 522-525.
- Hoerter, J. D., Bradley, P., Casillas, A., Chambers, D., Denholm, C., Johnson, K. and Weiswasser, B. (2012). Extrafollicular dermal melanocyte stem cells and melanoma. *Stem Cells Int.* **2012**, 407079.
- Hornby, S. J., Adolph, S., Gilbert, C. E., Dandona, L. and Foster, A. (2000). Visual acuity in children with coloboma: clinical features and a new phenotypic classification system. *Ophthalmology* **107**, 511-520.
- Hornyak, T. J., Hayes, D. J., Chiu, L. Y. and Ziff, E. B. (2001). Transcription factors in melanocyte development: distinct roles for Pax-3 and Mitf. *Mech. Dev.* **101**, 47-59.
- Hoth, C. F., Milunsky, A., Lipsky, N., Sheffer, R., Clarren, S. K. and Baldwin, C. T. (1993). Mutations in the paired domain of the human PAX3 gene cause Klein-Waardenburg syndrome (WS-III) as well as Waardenburg syndrome type I (WS-I). *Am. J. Hum. Genet.* **52**, 455-462.
- Hu He, K. H., Lorenzo, P. I., Brun, T., Jimenez Moreno, C. M., Aeberhard, D., Vallejo Ortega, J., Cornu, M., Thorel, F., Gjinovci, A., Thorens, B. et al. (2011). In vivo conditional Pax4 overexpression in mature islet  $\beta$ -cells prevents stress-induced hyperglycemia in mice. *Diabetes* **60**, 1705-1715.
- Huang, B., Pi, L., Chen, C., Yuan, F., Zhou, Q., Teng, J. and Jiang, T. (2012). WT1 and Pax2 re-expression is required for epithelial-mesenchymal transition in 5/6 nephrectomized rats and cultured kidney tubular epithelial cells. *Cells Tissues Organs* **195**, 296-312.
- Ishikiriyama, S. (1993). Gene for Waardenburg syndrome type I is located at 2q35, not at 2q37.3. *Am. J. Med. Genet.* **46**, 608.
- Jessen, K. R. and Mirsky, R. (2005). The origin and development of glial cells in peripheral nerves. *Nat. Rev. Neurosci.* **6**, 671-682.
- Jiang, Y. S., Jiang, T., Huang, B., Chen, P. S. and Ouyang, J. (2013). Epithelial-mesenchymal transition of renal tubules: divergent processes of repairing in acute or chronic injury? *Med. Hypotheses* **81**, 73-75.
- Jonas, J. B. and Freisler, K. A. (1997). Bilateral congenital optic nerve head pits in monozygotic siblings. *Am. J. Ophthalmol.* **124**, 844-846.
- Jostes, B., Walther, C. and Gruss, P. (1990). The murine paired box gene, Pax7, is expressed specifically during the development of the nervous and muscular system. *Mech. Dev.* **33**, 27-37.
- Kapadia, H., Frazier-Bowers, S., Ogawa, T. and D'Souza, R. N. (2006). Molecular characterization of a novel PAX9 missense mutation causing posterior tooth agenesis. *Eur. J. Hum. Genet.* **14**, 403-409.
- Kawabe, Y., Wang, Y. X., McKinnell, I. W., Bedford, M. T. and Rudnicki, M. A. (2012). *Carm1* regulates Pax7 transcriptional activity through MLL1/2 recruitment during asymmetric satellite stem cell divisions. *Cell Stem Cell* **11**, 333-345.
- Keller, C. and Guttridge, D. C. (2013). Mechanisms of impaired differentiation in rhabdomyosarcoma. *FEBS J.* **280**, 4323-4334.
- Keller, C., Arenkiel, B. R., Coffin, C. M., El-Bardeesy, N., DePinho, R. A. and Capecchi, M. R. (2004). Alveolar rhabdomyosarcomas in conditional Pax3:Fkhr mice: cooperativity of *Ink4a/ARF* and *Trp53* loss of function. *Genes Dev.* **18**, 2614-2626.
- Kioussi, C., Gross, M. K. and Gruss, P. (1995). Pax3: a paired domain gene as a regulator in PNS myelination. *Neuron* **15**, 553-562.
- Kist, R., Watson, M., Wang, X., Cairns, P., Miles, C., Reid, D. J. and Peters, H. (2005). Reduction of Pax9 gene dosage in an allelic series of mouse mutants causes hypodontia and oligodontia. *Hum. Mol. Genet.* **14**, 3605-3617.
- Kivlin, J. D., Fineman, R. M., Crandall, A. S. and Olson, R. J. (1986). Peters' anomaly as a consequence of genetic and nongenetic syndromes. *Arch. Ophthalmol.* **104**, 61-64.
- Koopitwut, S., Plengvidhya, N., Chukijrungrat, T., Sujitjoo, J., Semprasert, N., Furuta, H. and Yenchitsomanus, P. T. (2012). Defective PAX4 R192H transcriptional repressor activities associated with maturity onset diabetes of the young and early onset-age of type 2 diabetes. *J. Diabetes Complications* **26**, 343-347.
- Kredo-Russo, S., Mandelbaum, A. D., Ness, A., Alon, I., Lennox, K. A., Behlke, M. A. and Hornstein, E. (2012). Pancreas-enriched miRNA refines endocrine cell differentiation. *Development* **139**, 3021-3031.
- Krispin, S., Nitzan, E. and Kalcheim, C. (2010). The dorsal neural tube: a dynamic setting for cell fate decisions. *Dev. Neurobiol.* **70**, 796-812.
- Kwang, S. J., Brugger, S. M., Lazik, A. E., Wu, L. Y., Liu, Y. H., Ishii, M., Sangiorgi, F. O., Rauchman, M., Sucov, H. M. et al. (2002). *Mx2* is an immediate downstream effector of Pax3 in the development of the murine cardiac neural crest. *Development* **129**, 527-538.
- Lagha, M., Kormish, J. D., Rocancourt, D., Manceau, M., Epstein, J. A., Zaret, K. S., Relaix, F. and Buckingham, M. E. (2008). Pax3 regulation of FGF signaling affects the progression of embryonic progenitor cells into the myogenic program. *Genes Dev.* **22**, 1828-1837.
- Lammi, L., Halonen, K., Pirinen, S., Thesleff, I., Arte, S. and Nieminen, P. (2003). A missense mutation in PAX9 in a family with distinct phenotype of oligodontia. *Eur. J. Hum. Genet.* **11**, 866-871.
- Lang, D., Chen, F., Milewski, R., Li, J., Lu, M. M. and Epstein, J. A. (2000). Pax3 is required for enteric ganglia formation and functions with Sox10 to modulate expression of c-ret. *J. Clin. Invest.* **106**, 963-971.
- Lang, D., Lu, M. M., Huang, L., Engleka, K. A., Zhang, M., Chu, E. Y., Lipner, S., Skoutchji, A., Millar, S. E. and Epstein, J. A. (2005). Pax3 functions at a nodal point in melanocyte stem cell differentiation. *Nature* **433**, 884-887.
- Le, N., Nagarajan, R., Wang, J. Y., Araki, T., Schmidt, R. E. and Milbrandt, J. (2005). Analysis of congenital hypomyelinating Egr2Lo/Lo nerves identifies Sox2 as an inhibitor of Schwann cell differentiation and myelination. *Proc. Natl. Acad. Sci. USA* **102**, 2596-2601.
- Le Douarin, N. M., Calloni, G. W. and Dupin, E. (2008). The stem cells of the neural crest. *Cell Cycle* **7**, 1013-1019.
- Lechtenberg, R. and Ferretti, C. (1981). Ataxia with aniridia of Gillespie: a case report. *Neurology* **31**, 95-97.
- Lek, M., Dias, J. M., Marklund, U., Uhde, C. W., Kurdija, S., Lei, Q., Sussel, L., Rubenstein, J. L., Matisse, M. P., Arnold, H. H. et al. (2010). A homeodomain feedback circuit underlies step-function interpretation of a Shh morphogen gradient during ventral neural patterning. *Development* **137**, 4051-4060.

- Li, C. G. and Eccles, M. R. (2012). PAX Genes in Cancer; Friends or Foes? *Front. Genet.* **3**, 6.
- Li, J., Liu, K. C., Jin, F., Lu, M. M. and Epstein, J. A. (1999). Transgenic rescue of congenital heart disease and spina bifida in Splotch mice. *Development* **126**, 2495-2503.
- Liu, Y. (2004). Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention. *J. Am. Soc. Nephrol.* **15**, 1-12.
- Luan, Z., Liu, Y., Stuhlmiller, T. J., Marquez, J. and Garcia-Castro, M. I. (2013). SUMOylation of Pax7 is essential for neural crest and muscle development. *Cell. Mol. Life Sci.* **70**, 1793-1806.
- Macchia, P. E., Lapi, P., Krude, H., Pirro, M. T., Missero, C., Chiovato, L., Souabni, A., Baserga, M., Tassi, V., Pinchera, A. et al. (1998). PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. *Nat. Genet.* **19**, 83-86.
- Mackereth, M. D., Kwak, S. J., Fritz, A. and Riley, B. B. (2005). Zebrafish pax8 is required for otic placode induction and plays a redundant role with Pax2 genes in the maintenance of the otic placode. *Development* **132**, 371-382.
- Marquardt, T., Ashery-Padan, R., Andrejewski, N., Scardigli, R., Guillemot, F. and Gruss, P. (2001). Pax6 is required for the multipotent state of retinal progenitor cells. *Cell* **105**, 43-55.
- Mastick, G. S., Davis, N. M., Andrew, G. L. and Easter, S. S., Jr (1997). Pax-6 functions in boundary formation and axon guidance in the embryonic mouse forebrain. *Development* **124**, 1985-1997.
- Matsunaga, E., Araki, I. and Nakamura, H. (2000). Pax6 defines the diencephalic boundary by repressing En1 and Pax2. *Development* **127**, 2357-2365.
- Matsuo, T., Osumi-Yamashita, N., Noji, S., Ohuchi, H., Koyama, E., Myokai, F., Matsuo, N., Taniguchi, S., Doi, H., Iseki, S. et al. (1993). A mutation in the Pax-6 gene in rat small eye is associated with impaired migration of midbrain crest cells. *Nat. Genet.* **3**, 299-304.
- Mauvais-Jarvis, F., Smith, S. B., Le May, C., Leal, S. M., Gautier, J. F., Molokhia, M., Riveline, J. P., Rajan, A. S., Kevorkian, J. P., Zhang, S. et al. (2004). PAX4 gene variations predispose to ketosis-prone diabetes. *Hum. Mol. Genet.* **13**, 3151-3159.
- Mayanil, C. S., George, D., Freilich, L., Miljan, E. J., Mania-Farnell, B., McLone, D. G. and Bremer, E. G. (2001). Microarray analysis detects novel Pax3 downstream target genes. *J. Biol. Chem.* **276**, 49299-49309.
- Mayanil, C. S., Pool, A., Nakazaki, H., Reddy, A. C., Mania-Farnell, B., Yun, B., George, D., McLone, D. G. and Bremer, E. G. (2006). Regulation of murine TGFbeta2 by Pax3 during early embryonic development. *J. Biol. Chem.* **281**, 24544-24552.
- McCarroll, M. N., Lewis, Z. R., Culbertson, M. D., Martin, B. L., Kimelman, D. and Nechiporuk, A. V. (2012). Graded levels of Pax2a and Pax8 regulate cell differentiation during sensory placode formation. *Development* **139**, 2740-2750.
- McKinnell, I. W., Ishibashi, J., Le Grand, F., Punch, V. G., Addicks, G. C., Greenblatt, J. F., Dilworth, F. J. and Rudnicki, M. A. (2008). Pax7 activates myogenic genes by recruitment of a histone methyltransferase complex. *Nat. Cell Biol.* **10**, 77-84.
- Medic, S. and Ziman, M. (2010). PAX3 expression in normal skin melanocytes and melanocytic lesions (naevi and melanomas). *PLoS ONE* **5**, e9977.
- Meeus, L., Gilbert, B., Rydlewski, C., Parma, J., Roussie, A. L., Abramowicz, M., Vilain, C., Christophe, D., Costagliola, S. and Vassart, G. (2004). Characterization of a novel loss of function mutation of PAX8 in a familial case of congenital hypothyroidism with in-place, normal-sized thyroid. *J. Clin. Endocrinol. Metab.* **89**, 4285-4291.
- Mi, D., Carr, C. B., Georgala, P. A., Huang, Y. T., Manuel, M. N., Jeanes, E., Niisato, E., Sansom, S. N., Livesey, F. J., Theil, T. et al. (2013). Pax6 exerts regional control of cortical progenitor proliferation via direct repression of Cdk6 and hypophosphorylation of pRb. *Neuron* **78**, 269-284.
- Milet, C., Maczkowiak, F., Roche, D. D. and Monsoro-Burq, A. H. (2013). Pax3 and Zic1 drive induction and differentiation of multipotent, migratory, and functional neural crest in *Xenopus* embryos. *Proc. Natl. Acad. Sci. USA* **110**, 5528-5533.
- Moase, C. E. and Trasler, D. G. (1990). Delayed neural crest cell emigration from Sp and Spd mouse neural tube explants. *Teratology* **42**, 171-182.
- Monsoro-Burq, A. H., Wang, E. and Harland, R. (2005). Msx1 and Pax3 cooperate to mediate FGF8 and WNT signals during *Xenopus* neural crest induction. *Dev. Cell* **8**, 167-178.
- Mostowska, A., Biedziak, B. and Trzeciak, W. H. (2006). A novel mutation in PAX9 causes familial form of molar oligodontia. *Eur. J. Hum. Genet.* **14**, 173-179.
- Mullighan, C. G., Goorha, S., Radtke, I., Miller, C. B., Coustan-Smith, E., Dalton, J. D., Girtman, K., Mathew, S., Ma, J., Pounds, S. B. et al. (2007). Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* **446**, 758-764.
- Murdoch, B., DelConte, C. and Garcia-Castro, M. I. (2012). Pax7 lineage contributions to the mammalian neural crest. *PLoS ONE* **7**, e41089.
- Nakazaki, H., Reddy, A. C., Mania-Farnell, B. L., Shen, Y. W., Ichi, S., McCabe, C., George, D., McLone, D. G., Tomita, T. and Mayanil, C. S. (2008). Key basic helix-loop-helix transcription factor genes Hes1 and Ngn2 are regulated by Pax3 during mouse embryonic development. *Dev. Biol.* **316**, 510-523.
- Nakazaki, H., Shen, Y. W., Yun, B., Reddy, A., Khanna, V., Mania-Farnell, B., Ichi, S., McLone, D. G., Tomita, T. and Mayanil, C. S. (2009). Transcriptional regulation by Pax3 and TGFbeta2 signaling: a potential gene regulatory network in neural crest development. *Int. J. Dev. Biol.* **53**, 69-79.
- Nelson, J., Flaherty, M. and Grattan-Smith, P. (1997). Gillespie syndrome: a report of two further cases. *Am. J. Med. Genet.* **71**, 134-138.
- Nguyen, L. V., Vanner, R., Dirks, P. and Eaves, C. J. (2012). Cancer stem cells: an evolving concept. *Nat. Rev. Cancer* **12**, 133-143.
- Ninkovic, J., Steiner-Mezzadri, A., Jawerka, M., Akinci, U., Masserdotti, G., Petricca, S., Fischer, J., von Holst, A., Beckers, J., Lie, C. D. et al. (2013). The BAF complex interacts with Pax6 in adult neural progenitors to establish a neurogenic cross-regulatory transcriptional network. *Cell Stem Cell* **13**, 403-418.
- Nishimoto, K., Iijima, K., Shirakawa, T., Kitagawa, K., Satomura, K., Nakamura, H. and Yoshikawa, N. (2001). PAX2 gene mutation in a family with isolated renal hypoplasia. *J. Am. Soc. Nephrol.* **12**, 1769-1772.
- Nutt, S. L. and Busslinger, M. (1999). Monoallelic expression of Pax5: a paradigm for the haploinsufficiency of mammalian Pax genes? *Biol. Chem.* **380**, 601-611.
- Nutt, S. L., Vambrie, S., Steinlein, P., Kozmik, Z., Rolink, A., Weith, A. and Busslinger, M. (1999). Independent regulation of the two Pax5 alleles during B-cell development. *Nat. Genet.* **21**, 390-395.
- O'Donnell, F. E., Jr and Pappas, H. R. (1982). Autosomal dominant foveal hypoplasia and presenile cataracts. A new syndrome. *Arch. Ophthalmol.* **100**, 279-281.
- Olaopa, M., Zhou, H. M., Snider, P., Wang, J., Schwartz, R. J., Moon, A. M. and Conway, S. J. (2011). Pax3 is essential for normal cardiac neural crest morphogenesis but is not required during migration nor outflow tract septation. *Dev. Biol.* **356**, 308-322.
- Oliver, M. D., Dotan, S. A., Chemke, J. and Abraham, F. A. (1987). Isolated foveal hypoplasia. *Br. J. Ophthalmol.* **71**, 926-930.
- Opdecamp, K., Nakayama, A., Nguyen, M. T., Hodgkinson, C. A., Pavan, W. J. and Arnheiter, H. (1997). Melanocyte development in vivo and in neural crest cell cultures: crucial dependence on the Mitf basic-helix-loop-helix-zipper transcription factor. *Development* **124**, 2377-2386.
- Psat, A. and Rudnicki, M. A. (2012). The long, the short, and the micro: a polyA tale of Pax3 in satellite cells. *Cell Stem Cell* **10**, 237-238.
- Patel, S. R., Kim, D., Levitan, I. and Dressler, G. R. (2007). The BRCT-domain containing protein PTIP links PAX2 to a histone H3, lysine 4 methyltransferase complex. *Dev. Cell* **13**, 580-592.
- Patel, S. R., Ranghini, E. and Dressler, G. R. (2013). Mechanisms of gene activation and repression by Pax proteins in the developing kidney. *Pediatr. Nephrol.*
- Pearce, W. G., Mielke, B. W., Hassard, D. T., Climenhaga, H. W., Climenhaga, D. B. and Hodges, E. J. (1995). Autosomal dominant keratitis: a possible aniridia variant. *Can. J. Ophthalmol.* **30**, 131-137.
- Perrotti, D., Jamieson, C., Goldman, J. and Skorski, T. (2010). Chronic myeloid leukemia: mechanisms of blastic transformation. *J. Clin. Invest.* **120**, 2254-2264.
- Peters, H., Wilm, B., Sakai, N., Imai, K., Maas, R. and Balling, R. (1999). Pax1 and Pax9 synergistically regulate vertebral column development. *Development* **126**, 5399-5408.
- Pfeffer, P. L., Bouchard, M. and Busslinger, M. (2000). Pax2 and homeodomain proteins cooperatively regulate a 435 bp enhancer of the mouse Pax5 gene at the midbrain-hindbrain boundary. *Development* **127**, 1017-1028.
- Pfeffer, P. L., Payer, B., Reim, G., di Magliano, M. P. and Busslinger, M. (2002). The activation and maintenance of Pax2 expression at the mid-hindbrain boundary is controlled by separate enhancers. *Development* **129**, 307-318.
- Pfeifer, A., Courtney, M., Ben-Othman, N., Al-Hasani, K., Gjernes, E., Vieira, A., Druelle, N., Avolio, F., Faurite, B., Mansouri, A. et al. (2013). Induction of multiple cycles of pancreatic  $\beta$ -cell replacement. *Cell Cycle* **12**, 3243-3244.
- Poleev, A., Wendler, F., Fickenschner, H., Zannini, M. S., Yaginuma, K., Abbott, C. and Plachov, D. (1995). Distinct functional properties of three human paired-box-protein, PAX8, isoforms generated by alternative splicing in thyroid, kidney and Wilms' tumors. *Eur. J. Biochem.* **228**, 899-911.
- Pongpudpunth, M., Bhawan, J., Al-Natour, S. H. and Mahalingam, M. (2010). Nestin-positive stem cells in neurofibromas from patients with neurofibromatosis type 1-tumorigenic or incidental? *Am. J. Dermatopathol.* **32**, 574-577.
- Ramaesh, T., Collinson, J. M., Ramaesh, K., Kaufman, M. H., West, J. D. and Dhillon, B. (2003). Corneal abnormalities in Pax6<sup>+/</sup>- small eye mice mimic human aniridia-related keratopathy. *Invest. Ophthalmol. Vis. Sci.* **44**, 1871-1878.
- Ramaesh, T., Ramaesh, K., Leask, R., Springbett, A., Riley, S. C., Dhillon, B. and West, J. D. (2006). Increased apoptosis and abnormal wound-healing responses in the heterozygous Pax6<sup>+/</sup>- mouse cornea. *Invest. Ophthalmol. Vis. Sci.* **47**, 1911-1917.
- Relaix, F., Rocancourt, D., Mansouri, A. and Buckingham, M. (2005). A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. *Nature* **435**, 948-953.
- Rocheteau, P., Gayraud-Morel, B., Siegl-Cachedenier, I., Blasco, M. A. and Tajbakhsh, S. (2012). A subpopulation of adult skeletal muscle stem cells retains all template DNA strands after cell division. *Cell* **148**, 112-125.
- Roeb, W., Boyer, A., Cavenee, W. K. and Arden, K. C. (2007). PAX3-FOXO1 controls expression of the p57Kip2 cell-cycle regulator through degradation of EGR1. *Proc. Natl. Acad. Sci. USA* **104**, 18085-18090.
- Rolink, A., Nutt, S., Busslinger, M., ten Boekel, E., Seidl, T., Andersson, J. and Melchers, F. (1999). Differentiation, dedifferentiation, and redifferentiation of B-lineage lymphocytes: roles of the surrogate light chain and the Pax5 gene. *Cold Spring Harb. Symp. Quant. Biol.* **64**, 21-26.
- Rudnicki, M. A., Le Grand, F., McKinnell, I. and Kuang, S. (2008). The molecular regulation of muscle stem cell function. *Cold Spring Harb. Symp. Quant. Biol.* **73**, 323-331.
- Sansom, S. N., Griffiths, D. S., Faedo, A., Kleinyan, D. J., Ruan, Y., Smith, J., van Heyningen, V., Rubenstein, J. L. and Livesey, F. J. (2009). The level of the transcription factor Pax6 is essential for controlling the balance between neural stem cell self-renewal and neurogenesis. *PLoS Genet.* **5**, e1000511.
- Sarsfield, J. K. (1971). The syndrome of congenital cerebellar ataxia, aniridia and mental retardation. *Dev. Med. Child Neurol.* **13**, 508-511.

- Sato, T., Sasai, N. and Sasai, Y. (2005). Neural crest determination by co-activation of Pax3 and Zic1 genes in *Xenopus* ectoderm. *Development* **132**, 2355-2363.
- Schimmenti, L. A., Cunliffe, H. E., McNoe, L. A., Ward, T. A., French, M. C., Shim, H. H., Zhang, Y. H., Proesmans, W., Leys, A., Byerly, K. A. et al. (1997). Further delineation of renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations. *Am. J. Hum. Genet.* **60**, 869-878.
- Schnittger, S., Rao, V. V., Deutsch, U., Gruss, P., Balling, R. and Hansmann, I. (1992). Pax1, a member of the paired box-containing class of developmental control genes, is mapped to human chromosome 20p11.2 by in situ hybridization (ISH and FISH). *Genomics* **14**, 740-744.
- Schubert, F. R., Tremblay, P., Mansouri, A., Faisst, A. M., Kammandel, B., Lumsden, A., Gruss, P. and Dietrich, S. (2001). Early mesodermal phenotypes in splotch suggest a role for Pax3 in the formation of epithelial somites. *Dev. Dyn.* **222**, 506-521.
- Schwarz, M., Alvarez-Bolado, G., Dressler, G., Urbánek, P., Busslinger, M. and Gruss, P. (1999). Pax2/5 and Pax6 subdivide the early neural tube into three domains. *Mech. Dev.* **82**, 29-39.
- Seale, P., Sabourin, L. A., Girgis-Gabardo, A., Mansouri, A., Gruss, P. and Rudnicki, M. A. (2000). Pax7 is required for the specification of myogenic satellite cells. *Cell* **102**, 777-786.
- Serbedzija, G. N., Bronner-Fraser, M. and Fraser, S. E. (1994). Developmental potential of trunk neural crest cells in the mouse. *Development* **120**, 1709-1718.
- Shaham, O., Gueta, K., Mor, E., Oren-Giladi, P., Grinberg, D., Xie, Q., Cvekl, A., Shomron, N., Davis, N., Keydar-Prizant, M. et al. (2013). Pax6 regulates gene expression in the vertebrate lens through miR-204. *PLoS Genet.* **9**, e1003357.
- Shalom-Feuerstein, R., Serror, L., De La Forest Divonne, S., Petit, I., Aberdam, E., Camargo, L., Damour, O., Vigouroux, C., Solomon, A., Gaggioli, C. et al. (2012). Pluripotent stem cell model reveals essential roles for miR-450b-5p and miR-184 in embryonic corneal lineage specification. *Stem Cells* **30**, 898-909.
- Shaw, M. W., Falls, H. F. and Neel, J. V. (1960). Congenital Aniridia. *Am. J. Hum. Genet.* **12**, 389-415.
- Shimajiri, Y., Sanke, T., Furuta, H., Hanabusa, T., Nakagawa, T., Fujitani, Y., Kajimoto, Y., Takasu, N. and Nanjo, K. (2001). A missense mutation of Pax4 gene (R121W) is associated with type 2 diabetes in Japanese. *Diabetes* **50**, 2864-2869.
- Sisodiya, S. M., Free, S. L., Williamson, K. A., Mitchell, T. N., Willis, C., Stevens, J. M., Kendall, B. E., Shorvon, S. D., Hanson, I. M., Moore, A. T. et al. (2001). PAX6 haploinsufficiency causes cerebral malformation and olfactory dysfunction in humans. *Nat. Genet.* **28**, 214-216.
- Smith, C. A. and Tuan, R. S. (1994). Human PAX gene expression and development of the vertebral column. *Clin. Orthop. Relat. Res.* **302**, 241-250.
- Sobngwi, E. and Gautier, J. F. (2002). Adult-onset idiopathic Type I or ketosis-prone Type II diabetes: evidence to revisit diabetes classification. *Diabetologia* **45**, 283-285.
- Soleimani, V. D., Punch, V. G., Kawabe, Y., Jones, A. E., Palidwor, G. A., Porter, C. J., Cross, J. W., Carvajal, J. J., Kockx, C. E., van IJcken, W. F. et al. (2012). Transcriptional dominance of Pax7 in adult myogenesis is due to high-affinity recognition of homeodomain motifs. *Dev. Cell* **22**, 1208-1220.
- St-Onge, L., Sosa-Pineda, B., Chowdhury, K., Mansouri, A. and Gruss, P. (1997). Pax6 is required for differentiation of glucagon-producing alpha-cells in mouse pancreas. *Nature* **387**, 406-409.
- Stockton, D. W., Das, P., Goldenberg, M., D'Souza, R. N. and Patel, P. I. (2000). Mutation of PAX9 is associated with oligodontia. *Nat. Genet.* **24**, 18-19.
- Suter, D. M., Tirefort, D., Julien, S. and Krause, K. H. (2009). A Sox1 to Pax6 switch drives neuroectoderm to radial glia progression during differentiation of mouse embryonic stem cells. *Stem Cells* **27**, 49-58.
- Tassabehji, M., Read, A. P., Newton, V. E., Harris, R., Balling, R., Gruss, P. and Strachan, T. (1992). Waardenburg's syndrome patients have mutations in the human homologue of the Pax-3 paired box gene. *Nature* **355**, 635-636.
- Tavassoli, K., Rüger, W. and Horst, J. (1997). Alternative splicing in PAX2 generates a new reading frame and an extended conserved coding region at the carboxy terminus. *Hum. Genet.* **101**, 371-375.
- Tekin, M., Bodurtha, J. N., Nance, W. E. and Pandya, A. (2001). Waardenburg syndrome type 3 (Klein-Waardenburg syndrome) segregating with a heterozygous deletion in the paired box domain of PAX3: a simple variant or a true syndrome? *Clin. Genet.* **60**, 301-304.
- Tellier, A. L., Cormier-Daire, V., Abadie, V., Amiel, J., Sigaudy, S., Bonnet, D., de Lonlay-Debeney, P., Morrisseau-Durand, M. P., Hubert, P., Michel, J. L. et al. (1998). CHARGE syndrome: report of 47 cases and review. *Am. J. Med. Genet.* **76**, 402-409.
- Therault, F. M., Nuthall, H. N., Dong, Z., Lo, R., Barnabe-Heider, F., Miller, F. D. and Stefani, S. (2005). Role for Runx1 in the proliferation and neuronal differentiation of selected progenitor cells in the mammalian nervous system. *J. Neurosci.* **25**, 2050-2061.
- Ticho, B. H., Hilchie-Schmidt, C., Egel, R. T., Traboulsi, E. I., Howarth, R. J. and Robinson, D. (2006). Ocular findings in Gillespie-like syndrome: association with a new PAX6 mutation. *Ophthalmic Genet.* **27**, 145-149.
- Ton, C. C., Hirvonen, H., Miwa, H., Weil, M. M., Monaghan, P., Jordan, T., van Heyningen, V., Hastie, N. D., Meijers-Heijboer, H., Drechsler, M. et al. (1991). Positional cloning and characterization of a paired box- and homeobox-containing gene from the aniridia region. *Cell* **67**, 1059-1074.
- Tremblay, P., Dietrich, S., Mericskay, M., Schubert, F. R., Li, Z. and Paulin, D. (1998). A crucial role for Pax3 in the development of the hypaxial musculature and the long-range migration of muscle precursors. *Dev. Biol.* **203**, 49-61.
- Tuoc, T. C., Narayanan, R. and Stoykova, A. (2013). BAF chromatin remodeling complex: cortical size regulation and beyond. *Cell Cycle* **12**, 2953-2959.
- Underhill, D. A. (2012). PAX proteins and fables of their reconstruction. *Crit. Rev. Eukaryot. Gene Expr.* **22**, 161-177.
- Verhulst, S., Smet, H., Ceulemans, B., Geerts, Y. and Tassignon, M. J. (1993). Gillespie syndrome, partial aniridia, cerebellar ataxia and mental retardation in mother and daughter. *Bull. Soc. Belge Ophthalmol.* **250**, 37-42.
- Vilain, C., Rydlewski, C., Duprez, L., Heinrichs, C., Abramowicz, M., Malvaux, P., Renneboog, B., Parma, J., Costagliola, S. and Vassart, G. (2001). Autosomal dominant transmission of congenital thyroid hypoplasia due to loss-of-function mutation of PAX8. *J. Clin. Endocrinol. Metab.* **86**, 234-238.
- von Maltzahn, J., Jones, A. E., Parks, R. J. and Rudnicki, M. A. (2013). Pax7 is critical for the normal function of satellite cells in adult skeletal muscle. *Proc. Natl. Acad. Sci. USA* **110**, 16474-16479.
- Vorobyov, E., Mertsalov, I., Dockhorn-Dworniczak, B., Dworniczak, B. and Horst, J. (1997). The genomic organization and the full coding region of the human PAX7 gene. *Genomics* **45**, 168-174.
- Walcher, T., Xie, Q., Sun, J., Irmeler, M., Beckers, J., Öztürk, T., Niessing, D., Stoykova, A., Cvekl, A., Ninkovic, J. et al. (2013). Functional dissection of the paired domain of Pax6 reveals molecular mechanisms of coordinating neurogenesis and proliferation. *Development* **140**, 1123-1136.
- Wallin, J., Eibel, H., Neubüser, A., Wiltling, J., Koseki, H. and Balling, R. (1996). Pax1 is expressed during development of the thymus epithelium and is required for normal T-cell maturation. *Development* **122**, 23-30.
- Walther, C. and Gruss, P. (1991). Pax-6, a murine paired box gene, is expressed in the developing CNS. *Development* **113**, 1435-1449.
- Wang, Q., Kumar, S., Slevin, M. and Kumar, P. (2006). Functional analysis of alternative isoforms of the transcription factor PAX3 in melanocytes in vitro. *Cancer Res.* **66**, 8574-8580.
- Wang, Q., Kumar, S., Mitsios, N., Slevin, M. and Kumar, P. (2007). Investigation of downstream target genes of PAX3c, PAX3e and PAX3g isoforms in melanocytes by microarray analysis. *Int. J. Cancer* **120**, 1223-1231.
- Wang-Wuu, S., Soukup, S., Ballard, E., Gotwals, B. and Lampkin, B. (1988). Chromosomal analysis of sixteen human rhabdomyosarcomas. *Cancer Res.* **48**, 983-987.
- Ward, T. A., Nebel, A., Reeve, A. E. and Eccles, M. R. (1994). Alternative messenger RNA forms and open reading frames within an additional conserved region of the human PAX-2 gene. *Cell Growth Differ.* **5**, 1015-1021.
- White, R. B. and Ziman, M. R. (2008). Genome-wide discovery of Pax7 target genes during development. *Physiol. Genomics* **33**, 41-49.
- Wittig, E. O., Moreira, C. A., Freire-Maia, N. and Vianna-Morgante, A. M. (1988). Partial aniridia, cerebellar ataxia, and mental deficiency (Gillespie syndrome) in two brothers. *Am. J. Med. Genet.* **30**, 703-708.
- Wollnik, B., Tukel, T., Uyguner, O., Ghanbari, A., Kayserili, H., Emiroglu, M. and Yuksel-Apak, M. (2003). Homozygous and heterozygous inheritance of PAX3 mutations causes different types of Waardenburg syndrome. *Am. J. Med. Genet. A.* **122A**, 42-45.
- Yang, X. M. and Trasler, D. G. (1991). Abnormalities of neural tube formation in pre-spina bifida splotch-delayed mouse embryos. *Teratology* **43**, 643-657.
- Yang, Y., Chauhan, B. K., Cveklava, K. and Cvekl, A. (2004). Transcriptional regulation of mouse alphaB- and gammaF-crystallin genes in lens: opposite promoter-specific interactions between Pax6 and large Maf transcription factors. *J. Mol. Biol.* **344**, 351-368.
- Yu, W. M., Feltri, M. L., Wrabetz, L., Strickland, S. and Chen, Z. L. (2005). Schwann cell-specific ablation of laminin gamma1 causes apoptosis and prevents proliferation. *J. Neurosci.* **25**, 4463-4472.
- Zavadil, J., Bitzer, M., Liang, D., Yang, Y. C., Massimi, A., Kneitz, S., Piek, E. and Bottinger, E. P. (2001). Genetic programs of epithelial cell plasticity directed by transforming growth factor-beta. *Proc. Natl. Acad. Sci. USA* **98**, 6686-6691.
- Zhang, X., Huang, C. T., Chen, J., Pankratz, M. T., Xi, J., Li, J., Yang, Y., Lavaute, T. M., Li, X. J., Ayala, M. et al. (2010). Pax6 is a human neuroectoderm cell fate determinant. *Cell Stem Cell* **7**, 90-100.
- Zlotogora, J. (1995). X-linked albinism-deafness syndrome and Waardenburg syndrome type II: a hypothesis. *Am. J. Med. Genet.* **59**, 386-387.