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PAX GENES DURING NEURAL DEVELOPMENT AND THEIR POTENTIAL ROLE IN NEUROREGENERATION

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ABSTRACT

Pax genes encode a family of transcription factors that have long been recognised as obligate contributors to embryonic development of the CNS, with evidence obtained from various animal models illustrating phylogenetically conserved functions. Within the CNS, *Pax* genes play substantial roles in cellular and regional specification, proliferation, progenitor cell maintenance, anti-apoptosis and neural differentiation. This comprehensive review details the critical functions of those *Pax* genes involved in pre- and post-natal CNS development, provides possible molecular mechanisms by which *Pax* genes contribute to proliferation and differentiation of neuronal cells, and explains observed changes in *Pax* gene expression in response to neurotrauma in the mature animal.

Knowledge of the ability of individual *Pax* genes to specify precise lineages within the CNS is beneficial for cell replacement strategies, particularly in the production of "designer" cells for the treatment of neurodegenerative disorders. The manipulation of stem or committed cells so that they express definitive *Pax* genes may indeed assist in the pursuit of the holy grail of regenerative medicine – that of CNS cell replacement therapies leading to functional repair. We explain here, however, that only the sophisticated and precise use of *Pax* genes will lead to a successful outcome.

KEYWORDS

Brain; CNS repair; *Pax* genes; spinal cord; stem cell therapy; neuroregeneration

ABBREVIATIONS

bHLH, basic helix-loop-helix: cdk, cyclin-dependent kinases: CNS, central nervous system: GABA, gamma-aminobutyric acid: N-CAM, neural cellular adhesion molecule: Ng-CAM, neuron-glia cellular adhesion molecule: PSA-NCAM, polysialylated neural cellular adhesion molecule; SGZ, subgranular zone: SVZ, subventricular zone: TERT, Telomerase Reverse Transcriptase.

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1. INTRODUCTION

Central nervous system (CNS) repair remains an elusive target for biomedical research, due to the poor regenerative capacity and as yet intractable complexity of the CNS. Stem cell transplantation offers great promise for repair subsequent to neurodegenerative diseases, neurotrauma or maldevelopment. Treatment with replacement cells, however, will require in-depth knowledge of the genes/factors that control precursor cell development towards a fully functional, differentiated neural cell of a specific CNS region. Manipulation may be required

to direct cells along the appropriate neural lineage for replacement of lost or damaged cells for a specific CNS target region. The capacity of developmental genes to "prime" stem cells or modify their developmental pathways prior to transplantation is a tool currently being investigated by several laboratories worldwide (Berninger *et al.*, 2007; Cao *et al.*, 2005; Denham *et al.*, 2010; Heins *et al.*, 2002; Kayama *et al.*, 2009; Pera and Tam, 2010; Thomas *et al.*, 2009). As requisite orchestrators of CNS development, controlling cell specification from very early stages, *Pax* genes are likely candidates to augment cell transplantation therapies.

2. *PAX* GENES – DEVELOPMENT AND DIVERSITY

Pax genes encode multiple homologous Pax proteins which have all arisen from a single ancestral gene by gene duplication and mutation during evolution. *Pax* gene groups are defined by sequence homology, and more specifically by the presence, absence or modification of highly conserved structural domains in their encoded proteins (Balczarek *et al.*, 1997; Hadrys *et al.*, 2005; Vorobyov and Horst, 2006) (Figure 1). Subsequent species splitting and further gene duplication and modification within each group have resulted in nine vertebrate *Pax* genes (Balczarek *et al.*, 1997; Dahl *et al.*, 1997; Kay and Ziman, 1999; Treisman *et al.*, 1991; Walther and Gruss, 1991; Walther *et al.*, 1991; Ward *et al.*, 1994). Pax proteins are defined by the presence of a highly conserved N-terminal paired domain and a C-terminal transactivation domain, and may contain a conserved octapeptide encoding region and a full or partial homeodomain (Figure 2). Each *Pax* gene, in response to spatiotemporally varied environmental cues, produces alternate transcripts which encode alternate isoforms with distinct DNA binding specificities (Callaerts *et al.*, 1997; Kay and Ziman, 1999; Vogan and Gros, 1997; Wang *et al.*, 2007; Wang *et al.*, 2006; Ziman *et al.*, 1997; Ziman and Kay, 1998; Ziman *et al.*, 2001b) and alternate transactivation functions (Vogan and Gros, 1997; Vogan *et al.*, 1996; Walther and Gruss, 1991).

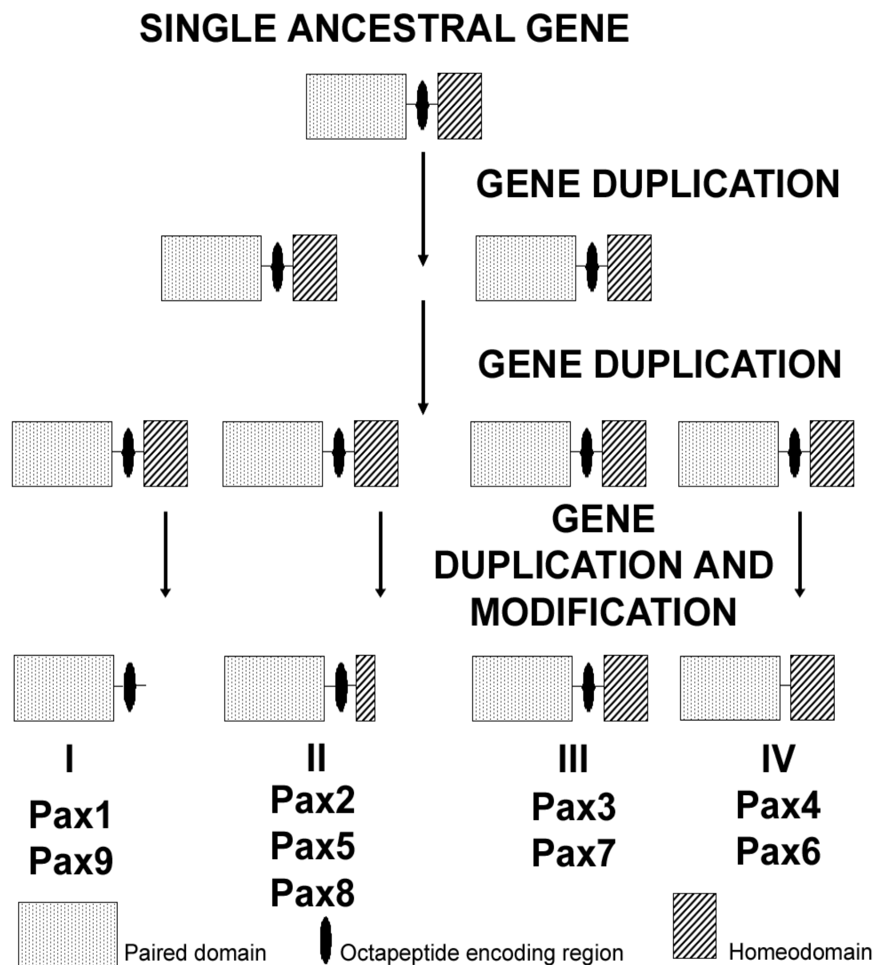

Figure 1. Diagram depicting the evolution of *Pax* genes – *Pax* genes are thought to have arisen from a single *Pax* ancestral gene which, through multiple gene duplications and domain modification, resulted in four homologous *Pax* genes all containing highly conserved paired DNA binding domains and may or may not include conserved octapeptide and variable homeodomain structures. Subsequent species splitting and gene duplication within groups have produced the nine currently identified vertebrate *Pax* genes (Adapted from Balczarek *et al.*, 1997; Hadrys *et al.*, 2005; Vorobyov and Horst, 2006).

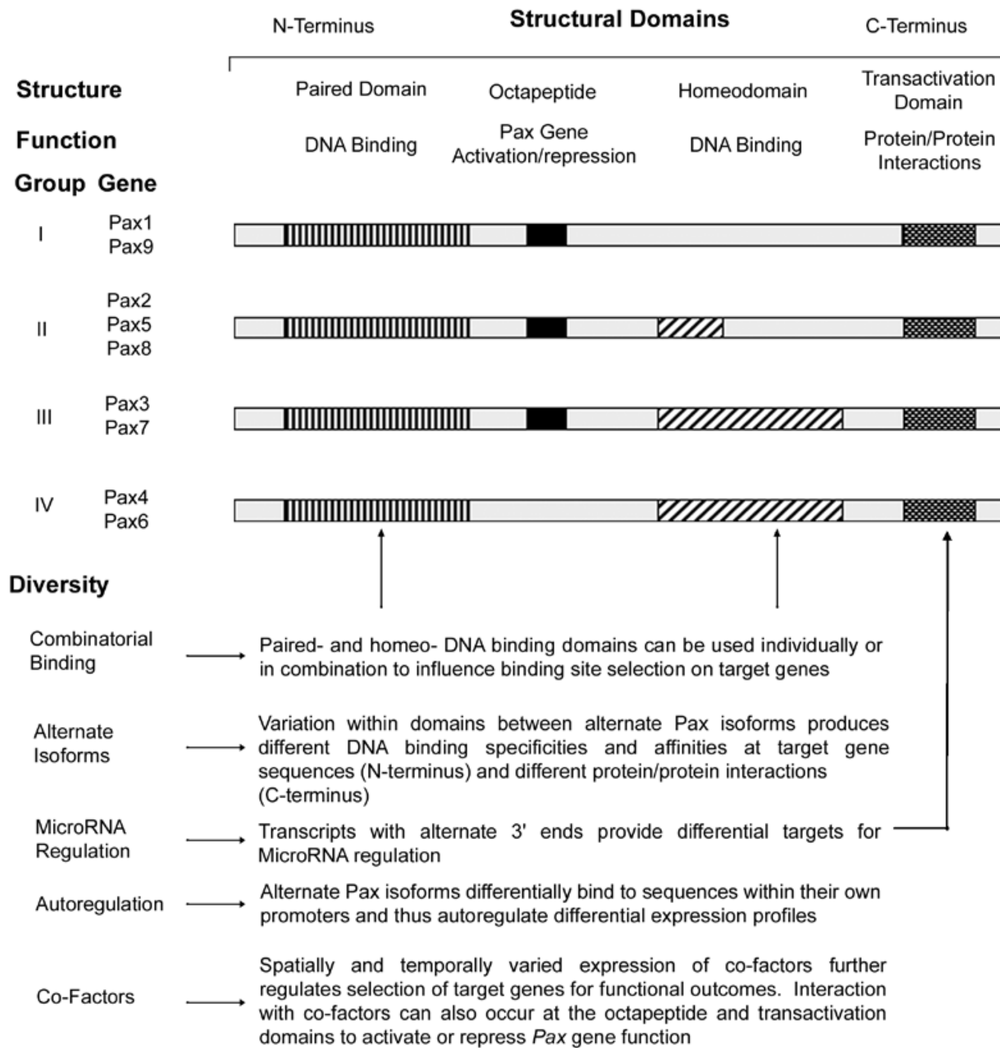

Figure 2 Thompson *Progress in Neurobiology*

Figure 2. Schematic of the structure of *Pax* Genes – *Pax* genes are defined by the presence of a paired box, which encodes a DNA binding domain containing 128 amino acids, and all contain a C-terminal transactivation domain. *Pax* gene subgroups are further differentiated based on the presence or absence of other structural regions, including a conserved octapeptide encoding region, and a partial or full DNA-binding homeodomain (60 amino acids). Further *Pax* gene diversity is achieved by encoding alternate N- and C-terminal isoforms with different DNA binding specificities and alternate microRNA regulation. Differential target gene selection is also varied by individual or combined use of DNA binding domains, together with spatial and temporal autoregulation, and participation of requisite co-factors.

Diversity of Pax gene function can be achieved using these isoforms by several mechanisms; individual or combined use of paired- or homeodomains for DNA binding site selection (Apuzzo and Gros, 2007; Underhill and Gros, 1997); microRNA regulation of 3' alternate gene transcripts (Chen *et al.*, 2010; Crist *et al.*, 2009; Dey *et al.*, 2011); alternate protein-protein interactions (Charytonowicz *et al.*, 2011); alternate DNA binding and transactivation in the presence of spatiotemporally varied co-factors (as detailed in section 5.2); spatial and temporal autoregulation (Grindley *et al.*, 1995; Plaza *et al.*, 1999) (Figure 2).

3. OVERVIEW OF *PAX* GENES IN CNS DEVELOPMENT

The *Pax* gene family displays dynamic spatiotemporal expression patterns and, together with other factors, act to co-ordinate regional CNS development, specifying neural subtypes and controlling their migration and differentiation. Expression studies and mutant models provide insight into their multiple developmental roles (Kawakami *et al.*, 1997; Lun and Brand, 1998; Mansouri *et al.*, 1996; Matsunaga *et al.*, 2000; Nomura *et al.*, 1998; Pfeffer *et al.*, 1998; Schwarz *et al.*, 1999; Thompson *et al.*, 2007; Thompson *et al.*, 2008). Notably, expression is not limited to embryogenesis; postnatal and adult expression is commonly observed (Hack *et al.*, 2005; Kawakami *et al.*, 1997; Kohwi *et al.*, 2005; Kukekov *et al.*, 1999; Maekawa *et al.*, 2005; Nacher *et al.*, 2005; Nakatomi *et al.*, 2002; Shin *et al.*, 2003; Thomas *et al.*, 2007; Thompson *et al.*, 2007). In this review, details of the substantive roles of *Pax* genes (specifically *Pax2, 3, 5, 6, 7* and *8*•) in CNS development are considered, from cell

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• *Pax1* and *Pax9* participate in vertebral column, bone, teeth, anterior digestive tract and thymus development (Gerber *et al.*, 2002; Neubuser *et al.*, 1995; Peters *et al.*, 1998; Wallin *et al.*, 1996) and *Pax4* is expressed in the pancreas (Brun *et al.*, 2004; Collombat *et al.*, 2009),

specification and regionalisation at early stages (Ericson *et al.*, 1997; Kawakami *et al.*, 1997; Matsunaga *et al.*, 2001; Nomura *et al.*, 1998; Schwarz *et al.*, 1999; Stoykova and Gruss, 1994) to proliferation, migration and differentiation at later stages (Burrill *et al.*, 1997; Chan-Ling *et al.*, 2009; Conway *et al.*, 2000; Kohwi *et al.*, 2005; Maekawa *et al.*, 2005; Marquardt *et al.*, 2001; Talamillo *et al.*, 2003). Throughout development, *Pax* gene expression holds subsets of cells in an anti-apoptotic, progenitor state until environmental stimuli dictate progression to proliferation or differentiation (Berger *et al.*, 2007; Kohwi *et al.*, 2005; Maekawa *et al.*, 2005; Nacher *et al.*, 2005; Underwood *et al.*, 2007; Yang *et al.*, 2008). The *Pax* family are therefore crucial in orchestrating and chaperoning maturing cells throughout multiple stages of CNS development and maturation.

4. *PAX* GENES TO ENHANCE CNS CELL REPLACEMENT THERAPY AND REPAIR

The ability to transplant replacement cells into the CNS to effect functional repair will ultimately depend upon knowledge of factors that direct embryonic stem cells along proliferation and neural differentiation processes to achieve formation and integration into tissue architecture and circuitry. Conditioning stem cells *in vitro* for cell replacement may require an accurate recapitulation of the neural milieu (Baizabal and Covarrubias, 2009), a difficult task considering the panoply of genes involved in regional CNS development and their highly dynamic spatiotemporal expression patterns. The accurate use of transcription factor combinations and concentrations to recapitulate cellular subtype specification would be extremely difficult to achieve *in vitro*. Therefore, a key gene at the top of a differential

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and whilst they do not participate in CNS development, they direct stem or progenitor cell specification within organs/tissues in which they are expressed.

hierarchy, such as the *Pax* genes, may well provide a solution to this problem. Furthermore, repair strategies for pathogenic or traumatic brain/spinal cord injury, or endogenous degeneration due to stroke/ischaemia, maldevelopment or neurodegeneration, will vary in the requirement of multiple cell types. This may be due to primary insult and secondary sequelae, or the requirement of restricted cell types for definitive cell replacement. The application of developmental *Pax* genes to produce a specific cell type or direct desired differentiation pathways may be beneficial for transplant therapies that require definitive cellular replacement. Examples include the *Pax6*-driven trans-differentiation of retinal pigmented epithelia into neuroretina for visual restoration (Arresta *et al.*, 2005; Azuma *et al.*, 2005), hair follicle stem cells into corneal epithelial-like cells for corneal repair (Yang *et al.*, 2009) and reprogramming of postnatal astroglia to a neuronal lineage (Berninger *et al.*, 2007; Heins *et al.*, 2002). Directed manipulation of a stem cell lineage appears to be an important step in cell transplantation protocols to reduce the possibility of host- or donor-derived tumour formation in the recipient (Amariglio *et al.*, 2009; Erdo *et al.*, 2003; Reubinoff *et al.*, 2001; Thomson *et al.*, 1998). A further consideration is how neuroinflammatory mechanisms operating within the regenerating tissue environment affect cell survival and maturation after transplant (reviewed in Jain, 2009; Park *et al.*, 2009). Taken together, these factors will affect the cell type and/or genetic manipulations required for successful therapeutic strategies. Whilst the exploitation of Pax transcription factors, or indeed that of any pivotal transcription factor, has great potential for regenerative purposes, complex and often dosage-dependent functions will require sophisticated and carefully considered use to ensure a successful outcome. In this paper our aim was to evaluate the capacity of *Pax* genes to enhance CNS cell replacement therapy and repair. To achieve this aim we first reviewed their developmental and regenerative capabilities and then assessed their demonstrated efficacy in cell lineage manipulation experiments drawing from *in vivo* and *in vitro* investigations. The

results presented below detail *Pax* gene function in initial regionalisation of the CNS, precursor cell specification and expansion, proliferation, migration, maintenance, and subsequent differentiation, and their capacity to withstand a post-insult environment. We also suggest mechanisms by which *Pax* genes may concomitantly regulate proliferation, stem cell maintenance and differentiation along specific cell lines. We took these results, together with those of recent stem cell replacement experiments, to formulate a considered opinion about the potential use of *Pax* genes for stem cell manipulation for CNS cell replacement therapies.

5. *PAX* GENES IN REGIONALISATION OF THE CNS

Pax gene expression occurs at the earliest stages of neural development, during gastrulation and neural plate formation. During neurulation, anteroposterior and dorsoventral signalling centres pattern the CNS, culminating in distinct gene expression domains that cause regional subdivision (Lumsden and Krumlauf, 1996; Redies and Puelles, 2001). *Pax* genes are key mediators of this process, differentially responding to signalling molecules (Crossley *et al.*, 1996; Ericson *et al.*, 1996; Ericson *et al.*, 1997; Fedtsova *et al.*, 2008; Fogel *et al.*, 2008; Joyner, 1996; Liem *et al.*, 1995; Monsoro-Burq *et al.*, 1996) thus contributing to cell type specification and brain regionalisation (Burrill *et al.*, 1997; Kawakami *et al.*, 1997; Nomura *et al.*, 1998; Schwarz *et al.*, 1999; Soukkarieh *et al.*, 2007). Graded *Pax* expression results from the spatial proximity of the *Pax*-expressing cells to the signalling centres and, as will be discussed later, differential Pax levels contribute to cellular diversity, a mechanism commonly used during development to produce cell type variation.

5.1 Dorsoventral Patterning of the CNS

Pax3 and *Pax7* are expressed at the dorsal edges of the early neural plate preceding neural tube closure (Basch *et al.*, 2006; Otto *et al.*, 2006), where they dorsalise cells along the entire neural tube. Cells of the dorsal neural tube form sensory neurons and interneurons (Goulding *et al.*, 1991; Jostes *et al.*, 1991), as well as neural crest cells, all of which require *Pax3/7* (Auerbach, 1954; Bang *et al.*, 1997; Basch *et al.*, 2006; Goulding *et al.*, 1991; Otto *et al.*, 2006). *Pax6* expression occurs along the entire mid-ventral region of the developing neural tube, generating motor neurons and interneurons (Goulding *et al.*, 1993). *Pax2* expression within the neural tube occurs at the intermediate dorsoventral boundary of the rhombencephalon and spinal cord (Nornes *et al.*, 1990), producing hindbrain and spinal cord interneurons (Burrill *et al*., 1997; Ponti *et al*., 2008).

5.2 Anteroposterior Patterning of the CNS

Highly specific *Pax*-directed anterior-posterior patterning during regionalisation of the developing CNS has been demonstrated by expression and transgenic studies for multiple *Pax* genes (Kawakami *et al.*, 1997; Matsunaga *et al.*, 2000, 2001; Nomura *et al.*, 1998; Schwarz *et al.*, 1999; Stoykova and Gruss, 1994). Initially, differential *Pax6 and Pax2* expression subdivides the neural tube into three primary anteroposterior domains (prosencephalon, mesencephalon and rhombencephalon) (Matsunaga *et al.*, 2000; Nornes *et al.*, 1990; Schwarz *et al.*, 1999). Subsequent to this, and as detailed in Table 1, the repressive relationship between *Pax* genes, or between *Pax* and other genes, participates in

determination of polarity, boundary formation and progenitor cell specification from ventricular zones (VZ) within these regions to form brain nuclei and associated structures.

The ability of *Pax* genes to mutually repress expression of alternate group *Pax* genes leads to zones of exclusivity for each *Pax* gene or group of *Pax* genes (Table 1). Pax proteins may

also cooperate with other transcription factors (eg opposing gradients of Pax6 and Emx2 or Pax6 and Dlx2 in cerebral cortex, and Pax6 and Olig2 in olfactory bulb/Pax6 with cVax and Tbx5 in retina) to achieve specification of cellular subtypes, boundary formation and arealisation (Bishop *et al.*, 2000; Brill *et al.*, 2008; Hack *et al.*, 2005; Hamasaki *et al.*, 2004; Leconte *et al.*, 2004; Muzio and Mallamaci, 2003).

Complex *Pax* activity is highly co-ordinated to achieve differential regulatory mechanisms at different times and in different locations. For instance, the co-operative and redundant activity of *Pax6* and *Pax2* specifies retinal pigmented epithelia at early optic vesicle stages (Baumer *et al.*, 2003) whilst they are mutually repressive at later optic cup stages (Baumer *et al.*, 2002; Schwarz *et al.*, 2000). Correspondingly, mutual co-ordinated repression between *Pax6* and *Pax2/5/8* regulates development of spinal cord interneurons (Bel-Vialar *et al.*, 2007; Burrill *et al.*, 1997; Pillai *et al.*, 2007). Moreover, *Pax3* and *Pax7* co-ordinate neurogenesis within the midbrain, evidenced by altered *Pax3* expression in *Pax7*$^{-/-}$ mutant mice (Thompson *et al*., 2008), and *Pax7* upregulation in *Pax3* hypomorphic mice (Zhou *et al.*, 2008). Thus, synchronous and highly co-ordinated *Pax* expression is critical for stipulating early patterning processes during CNS development.

Table 1. Differential *Pax* expression during anteroposterior patterning.

Region	*Pax* Gene	Expression and Potential Function	References
Forebrain	*Pax2*	Forebrain/midbrain boundary (with En-1) (opposes *Pax)*	(Matsunaga *et al.*, 2000; Schwarz *et al.*, 1999)
		Specification of precursor cells of the forebrain, eye field, otic vesicle	(Fotaki *et al.*, 2008; Nornes *et al.*, 1990; Torres *et al.*, 1996)
	Pax6	Forebrain/midbrain boundary formation (opposes *Pax2)*	(Matsunaga *et al.*, 2000; Schwarz *et al.*, 1999)
		Specification of cortical progenitors, and cortical arealisation (opposes *Emx2)*	(Bishop *et al.*, 2000; Gotz *et al.*, 1998)
		Specification of neural plate cells of the eye field/dorsoventral patterning of the retina	(Leconte *et al.*, 2004; Zaghloul and Moody, 2007)
	Pax7	Specification of hypothalamic progenitor cells	(Ohyama *et al.*, 2008)
Midbrain	*Pax 2/5/8*	Midbrain/hindbrain boundary formation and polarisation regulated by the isthmic organiser	(Brand *et al.*, 1996; Lun and Brand, 1998; Pfeffer *et al.*, 1998; Picker *et al.*, 1999; Rowitch and McMahon, 1995)
	Pax6	Expressed in ventral embryonic midbrain and adult dorsolateral substantia nigra reticularis	(Stoykova and Gruss, 1994)
	Pax 3	Specification of the dorsal mesencephalon and is restricted to undifferentiated mesencephalic cells after boundary formation	(Matsunaga *et al.*, 2001; Stoykova and Gruss, 1994; Thompson *et al.*, 2008)
	Pax7	Specification of the dorsal mesencephalon, and is expressed in undifferentiated and differentiated (neuronal) mesencephalic cells after boundary formation	(Kawakami *et al.*, 1997; Matsunaga *et al.*, 2001; Nomura *et al.*, 1998; Stoykova and Gruss, 1994; Thompson *et al.*, 2007; Thompson *et al.*, 2008)
		Polarisation of the dorsal mesencephalon	(Thomas *et al.*, 2007; Thompson *et al.*, 2007)

<table>
<tr><td rowspan="5">Hindbrain</td><td rowspan="2">Pax
2/5/8</td><td>Participates in midbrain/hindbrain boundary formation</td><td>(Brand et al., 1996; Lun and Brand, 1998; Pfeffer et al., 1998; Picker et al., 1999; Rowitch and McMahon, 1995)</td></tr>
<tr><td>Specification and differentiation of GABAergic interneurons</td><td>(Maricich and Herrup, 1999)</td></tr>
<tr><td>Pax6</td><td>Expressed in mouse ventricular zone and external germinative layer. Controls differentiation and proliferation of motor neurons and ventral interneurons from ventral progenitors</td><td>(Ericson et al., 1997; Stoykova and Gruss, 1994)</td></tr>
<tr><td>Pax3</td><td>Specifies rhombomeric caudal neural crest cells Expressed in ventricular zone of mouse hindbrain</td><td>(Goulding et al., 1991; Mansouri et al., 1996; Stoykova and Gruss, 1994)</td></tr>
<tr><td>Pax7</td><td>Specifies rhombomeric caudal neural crest cells. Expressed around Purkinje cells (cerebellum) – may relate to maintenance of normal physiology</td><td>(Mansouri et al., 1996; Shin et al., 2003; Stoykova and Gruss, 1994)</td></tr>
<tr><td rowspan="2">Spinal Cord</td><td>Pax2</td><td>Specification and maintenance of GABAergic cells of dorsal horn interneurons (inhibiting Pax6)</td><td>(Bel-Vialar et al., 2007; Burrill et al., 1997; Pillai et al., 2007)</td></tr>
<tr><td>Pax6</td><td>Development of spinal interneurons (inhibiting Pax2/5/8)</td><td>(Bel-Vialar et al., 2007; Burrill et al., 1997; Pillai et al., 2007)</td></tr>
</table>

The critical importance of *Pax* genes in regionalisation and cellular specification is exemplified by mutant animal models, where absence of Pax transcription factors results in patterning abnormalities and loss of cells and structures (Table 2).

Moreover, conditional mutant models (knockout/reduced/overexpression) have allowed an intricate dissection of Pax function at variable stages of development, without initial ablation of structures, lack of initial proliferation or lethality issues. It is evident that analyses of such valuable animal models will permit a deeper understanding of the temporospatial influence of *Pax* genes during various stages of development (Manuel *et al.*, 2007; Marquardt *et al.*, 2001; Pinon *et al.*, 2008; Tuoc *et al.*, 2009), and they demonstrate the requisite nature of *Pax* genes in correct cellular specification and patterning.

Table 2. Patterning and structural abnormalities in *Pax* mutant animal models.

***Pax* Gene**	**Patterning and structural abnormalities**	**References**
Pax2	**Loss of the isthmus;** failure to close the midbrain neural tube, abnormalities of the midbrain/hindbrain region	(Brand *et al*., 1996; Torres *et al*., 1996)
	Visual defects; alteration to the optic nerve projection and formation of the retina/optic nerve boundary; agenesis of the optic chiasm and failure to close the optic fissure	(Torres *et al.,* 1996)
	Auditory defects; abnormal epithelial morphogenesis producing defects of the cochlear and spiral ganglion of the inner ear	(Christophorou *et al.*, 2010; Torres *et al.*, 1996)
Pax5	**Midbrain defects;** reduction of the inferior colliculus	(Urbanek *et al.,* 1994)
	Hindbrain defects; altered foliation of the anterior cerebellum	
Pax6	**Cortical defects;** Thalamocortical and corticofugal axonal pathfinding errors in *Pax6* null mutants; no evidence in *Pax6* conditional knockout/overexpression models Cortical layering abnormalities and premature differentiation of late-born cortical progenitors; ventralisation of dorsal telencephalic progenitors in *Pax6* null mice with subsequent ectopic GABA interneuron formation	(Jones *et al*., 2002; Kawano *et al*., 1999; Kroll and O'Leary, 2005; Manuel *et al.*, 2007; Pinon *et al.*, 2008; Pratt *et al.*, 2000; Pratt *et al.*, 2002; Tuoc *et al.*, 2009)
	Visual defects; Microphthalmia (*Pax6* over-/under-expression) or anophthalmia (absence of *Pax6*); dorsalisation of the retina (*Pax6* overexpression), ventralisation of the retina (absence of *Pax6*)	(Baumer *et al*., 2002; Hill *et al.,* 1991; Hogan *et al.,* 1986; Leconte *et al.*, 2004; Manuel *et al.*, 2007)
	Craniofacial defects; Required for differentiation of nasal placodes; *Pax6* null mice exhibit an imperforate snout	(Hill *et al*., 1991; Hogan *et al*., 1986)
Pax2/ Pax6#	**Visual defects;** Transdifferentiation of retinal pigmented epithelium into neuroretina	(Baumer *et al*., 2003)
Pax3	**Neural tube defects;** Open neural folds, neural tube irregularities and exencephaly (Embryonic lethal)	(Auerbach, 1954)
	Neural crest defects; Cardiac defect - conotruncal defect due to reduction in migratory cardiac neural crest cells	(Conway *et al*., 2000)
Pax7	**Midbrain defects;** Failure to maintain a subpopulation of dorsal mesencephalic neurons	(Thompson *et al.*, 2008)
	Craniofacial defects; Absence of nasal capsule and reduction in maxilla and tubules of nasal serous glands due to aberrant neural crest cell specification	(Mansouri *et al*., 1996)
Pax3/ Pax7#	**Neural tube and spinal cord defects;** extensive exencephaly/spina bifida/ventralisation of dorsal spinal cord interneurons (Embryonic lethal)	(Mansouri and Gruss, 1998)

6. *PAX* GENES IN PROGENITOR CELL EXPANSION AND MAINTENANCE

After brain regionalisation, the contribution of *Pax* genes to the intricate balance between cell proliferation, progenitor cell maintenance and differentiation (and therefore in CNS growth and development) has been demonstrated by substantial research.

For instance, *Pax6* controls progenitor pool expansion, often in a dosage-dependent manner, in developing regions such as the optic vesicle (Duparc *et al.*, 2007), the cerebral cortex (Berger *et al.*, 2007; Estivill-Torrus *et al.*, 2002; Sansom *et al.*, 2009; Tuoc *et al.*, 2009) and the postnatal hippocampus (Maekawa *et al.*, 2005; Nacher *et al.*, 2005). Within the eye field *Pax6* similarly promotes proliferation of retinal stem cells, expanding the proliferative pool from early optic vesicle stages and throughout multiple stages of retinogenesis (*Xenopus*) (Zaghloul and Moody, 2007). Reduced Pax6 levels lead to reduced proliferation and/or precocious differentiation of neurogenic precursors in the eye (Duparc *et al.*, 2007; Philips *et al.*, 2005), cerebral cortex (Tuoc *et al.*, 2009) developing spinal cord (Bel-Vialar *et al.*, 2007), and reduced proliferation in the postnatal hippocampus (Maekawa *et al*., 2005). In contrast, overexpression of *Pax6* reduces proliferation of late-born cortical progenitors, demonstrating the differential dosage sensitivity of this cellular subpopulation (Tuoc *et al*., 2009). These results demonstrate that Pax6 levels mediate the critical spatiotemporal orchestration of progenitor cell proliferation and differentiation to produce precise CNS regions.

Likewise, *Pax3* is also necessary for progenitor expansion and for maintenance of the undifferentiated phenotype. Premature neurogenesis is observed at E10.0 in the lumbar neural tube of *Pax3*$^{-/-}$ mice and in the neural tube explant cultures from these mice (Nakazaki *et al.*, 2008); moreover, *Pax3* expression within the developing superior colliculus (dorsal

mesencephalon) is restricted to undifferentiated neural precursor cells and disappears once the cells differentiate (Thompson *et al.*, 2008). In cultured mouse neuroblastoma cells, downregulation of *Pax3* by antisense RNA leads to differentiation of cells into mature neurons (Reeves *et al.*, 1999). Similarly, neural crest cell development provides an exquisite example of the capacity of *Pax3* to regulate progenitor cell expansion and maintenance of an undifferentiated phenotype. *Pax3*-expressing neural crest cells initially arise in the dorsal neural tube. Once committed by a number of transcription factors, including Pax3, along Schwann as well as melanocytic or cardiac lineages, the cells proliferate and migrate as undifferentiated cells to populate the peripheral nervous system (Schwann cells) (Kioussi *et al.*, 1995), skin (melanocytes) (Bang *et al.*, 1997; Blake and Ziman, 2005; Goulding *et al.*, 1991; Hornyak *et al.*, 2001), and heart (cardiac) (Conway *et al.*, 2000). *Pax3* is expressed throughout their specification, migration and differentiation. In fact neural crest cell-derived precursors require Pax3; in *Pax3*$^{-/-}$ mice these cells undergo normal migratory and survival functions but have reduced progenitor expansion resulting in developmental defects (Conway *et al.*, 2000). *Pax7* also specifies a subpopulation of mouse cephalic neural crest cells that migrate to the craniofacial region (Mansouri *et al.*, 1996) and eventually give rise to a wide variety of olfactory epithelial cell types (Murdoch *et al.*, 2010). Similarly, *Pax7*$^{-/-}$ mutant mice exhibit craniofacial abnormalities (Mansouri *et al.*, 1996). Interestingly, whilst *Pax3* expression occurs in both premigratory and migrating neural crest cells, *Pax7* expression is restricted to migrating neural crest cells (Betters *et al.*, 2010), indicating divergence during early (premigratory) functions.

One mechanism by which Pax proteins control progenitor expansion and maintenance involves their regulation of distinct downstream targets, for example cell cycle regulators. Pax3 activates progenitor expansion within the neural tube and forebrain by activating *Hes1*,

which represses *p21*^{cip}, a cell cycle regulator known to promote quiescence of neural progenitor proliferation (Kippin *et al*., 2005; Nakazaki *et al.*, 2008). Moreover, *Hes1/Hes3* compound mutant mice phenocopy *Pax2/Pax5* mutant mice (absence of midbrain and anterior hindbrain structures), with premature termination of *Pax2/5/8* expression, and loss of isthmic organiser activity, due to an inability to maintain isthmic ventricular cells. This lack of progenitor maintenance results in premature neuronal differentiation, a common feature of downregulated *Pax* expression. This result together with other studies suggests a regulatory relationship between *Hes1/3* and *Pax2/5/8* genes to bring about a delay in the neurogenesis of isthmic cells, thereby maintaining isthmic organiser activity (Hirata *et al*., 2001).

Notably, the ability of Pax transcription factors to bind to cell cycle regulators, thus controlling proliferation versus differentiation, is also demonstrated by direct binding of Pax6 to genes involved in stem cell self-renewal (eg *Hmga2*), cell cycle progression and proliferation (e.g. Pten and cyclin-dependent kinases (Cdk4)), neuronal cell cycle inhibition (*Hes5/6*), and neuronal differentiation (*Ngn2*) (Sansom *et al.*, 2009; Scardigli *et al.*, 2003). These results identify some of the mechanisms and downstream targets whereby *Pax* genes regulate the switch from cell proliferation to differentiation.

7. *PAX* GENES IN CELLULAR MIGRATION

A key feature of organogenesis is the orchestrated temporospatial migration of cells, which appears to be of pivotal importance for conditional specification of correct cellular subclasses. *Pax* genes contribute extensively to cellular migration, as shown in Table 3.

Table 3: Examples of *Pax*–expressant neural migratory cells

***Pax* Gene**	**Migrating population/tissue**	**References**
Pax2	Expressed in precursor and immature astrocytic cells migrating within the retina, and in migrating interneurons of the postnatal cerebellum and ventral spinal cord	(Burrill *et al.*, 1997; Chan-Ling *et al.*, 2009; Ponti *et al.*, 2008)
Pax6	Expressed in migrating neuronal precursors, neurons and interneurons within the developing cerebrum and medulla oblongata, and adult cerebellum	(Caric *et al.*, 1997; Horie *et al.,* 2003; Jimenez *et al.,* 2002; Mo and Zecevic, 2008; Ponti *et al.*, 2008; Talamillo *et al.*, 2003)
	Expressed in migrating neuroblasts within adult neurogenic regions of the dentate gyrus of the hippocampus and rostral migratory stream to the olfactory bulb	(Hack *et al*., 2005; Kohwi *et al*., 2005; Maekawa *et al*., 2005; Nacher *et al*., 2005)
	Adult rat spinal cord after trauma	(Yamamoto *et al*., 2001)
Pax3	Neural crest cells migrate to cephalic mesenchyme, skin, peripheral nervous system and heart	(Betters *et al*., 2010; Conway *et al*., 2000; Goulding *et al.*, 1991; Hornyak *et al.*, 2001; Kioussi *et al*., 1995)
Pax7	Neural crest cells migrate to craniofacial regions, and neuroblasts migrate within the midbrain to form the laminated structure of the superior colliculus	(Betters *et al.*, 2010; Mansouri *et al.*, 1996; Murdoch *et al.*, 2010)

For instance, *Pax3*-expressing neural crest cells migrate extensively throughout the body and Pax-3 deficiency results in altered migration or reduced cells at the target destination (Hornyak *et al*., 2001; Nakazaki *et al*., 2008). *Pax6* is also required for correct cellular migration in some cell populations, and *Pax6*-deficiency can result in altered migration of neuroblasts in the developing mouse cerebral cortex (Jimenez *et al*., 2002; Talamillo *et al*., 2003) and in the medullary cerebellum (Horie *et al*., 2003). A reduction of cells at the target destination, however, may not always be due to a migratory deficit, but rather a failure to adequately expand the progenitor pool. Moreover, expression during migration does not necessarily infer causality in this process; it may be that *Pax* gene expression is required for

maintaining progenitor status during migration to provide cells for proliferation at new organs/tissue.

Evidence of a role for Pax proteins in influencing migratory capacity can be discerned from their interactions (direct or indirect) with genes such as cellular adhesion molecules, and this feature is also differentially affected by alternate isoforms acting on distinct downstream targets (Wang *et al.*, 2008; Wang *et al.*, 2007; Zhang *et al.*, 2010). *Pax* paired box DNA binding sites have been discovered in the promoters of several neural cell adhesion molecules (neural cell adhesion molecule (N-CAM), neuron-glia cell adhesion molecule (Ng-CAM) and L1). Transfection experiments in a variety of cell lines show that these sites are regulated by Pax1, Pax3, Pax6 and Pax8 (Edelman and Jones, 1998); Pax6 has also been shown to regulate expression of L1 *in vivo* (Meech *et al*., 1999) and L1 expression is abnormal in *Pax6*$^{-/-}$ mice (Caric et al., 1997). Maekawa *et al* (2005) also demonstrated colocalised expression of *Pax6* and *polysialylated N-CAM (PSA-NCAM)* in cells of the postnatal rat dentate gyrus. Furthermore, Wang *et al* (2008) detected upregulated *Met* and *Muc18* (mRNA and protein) in melanocytes transfected with the Pax3c isoform. Pax3 also regulates *c-Met* during muscle precursor migration (Epstein *et al*., 1996; Mayanil *et al*., 2001), and increased polysialylation of N-CAM due to *Pax3* overexpression is observed in a medulloblastoma cell line (Mayanil *et al*., 2000).

Discernment of the cell-autonomous and non-autonomous contribution of *Pax* genes to migratory capacity has been achieved through transplantation of *Pax*-deficient cells into a normal host environment, and vice versa (Kohwi *et al.*, 2005; Osumi-Yamashita *et al.*, 1997). Unambiguous identification of a *Pax* role in non-cell-autonomous migration comes from studies where midbrain-derived neural crest cells (which do not express *Pax6*) do not migrate

appropriately to the eye (Kanakubo *et al*., 2006) and craniofacial region (Osumi-Yamashita *et al*., 1997) in *Pax6*-deficient rats. Transplantation of midbrain-derived neural crest cells from wildtype rats into the *Pax6*-deficient environment does not rescue migration, indicating that the fault occurs due to an incorrectly specified migratory pathway and not due to deficits in the migrating cell (Osumi-Yamashita *et al*., 1997). In support of this, *Pax6*$^{-/-}$ late born cortical precursor cells transplanted into a wildtype environment showed similar migratory capacity to wildtype cells, indicating that *Pax6* does not bestow a cell-autonomous migratory capacity to the cell in this instance (Caric *et al.,* 1997).

Pax genes also contribute to migratory processes within a developing tissue, such as in axon guidance (Jones *et al*., 2002; Kanakubo *et al*., 2006; Kawano *et al*., 1999; Osumi-Yamashita *et al*., 1997; Pratt *et al*., 2002). During cortical development, progenitors from the SVZ migrate to their appropriate destination, using PSA-NCAM and robo2 as guidance molecules. *Pax6* mutant mice exhibit qualitative changes to PSA-NCAM+ tracts within the intermediate zone, disrupted (delayed and downregulated) expression of robo2 and subsequent migratory deficits (Jimenez *et al*., 2002). Moreover, *Pax6*-expressing cells of the foetal rat medulla oblongata associate with the neural cell adhesion molecule TAG1, and migrate along *TAG1*-expressing axons. In this region of *Pax6*$^{-/-}$ rats, *TAG1* expression is delayed and a subpopulation of these cells migrate aberrantly (Horie *et al*., 2003). Within the rostral migratory stream, Pax6-positive neuroblasts migrate tangentially toward the olfactory bulb, whereby migration halts, neuroblasts detach and then migrate radially to the olfactory bulb. Tenascin-R is an extracellular matrix molecule which fosters neuroblast detachment and radial migration; tenascin-R-deficient mice exhibit altered migration of olfactory neuroblasts (Saghatelyan *et al*., 2004). Biochemical evidence of a direct relationship between Tenascin-R and Pax proteins during migration, however, has not been demonstrated to date.

Pax2 regulation of the cellular adhesion molecules N-CAM and N-cadherin, although more related to morphogenesis than migration, is also elegantly demonstrated in investigation of chick otic development; morpholino knockdown of *Pax2* results in absence of the above named molecules, whilst *Pax2* overexpression results in their upregulation, and ectopic Pax2 induces their expression (Christophorou *et al.*, 2010).

This review of Pax endowment of migratory capacity, whilst not exhaustive in nature, indicates a complex contribution of *Pax* genes towards directed migration of cells in both embryonic and postnatal environments. This feature may be manipulated to deliver cells to an appropriate destination, or to block migration of transplanted cells.

8. *PAX* GENES IN CELL FATE SPECIFICATION

After CNS regionalisation, *Pax* expression becomes increasingly restricted as cellular specification proceeds. In fact a recognised feature of *Pax* genes is their ability to act as a functional switch between progenitor maintenance and differentiation. For example, as eye development progresses, *Pax6* functions to switch neuroepithelial cells of the mouse optic vesicle from proliferation towards differentiation. At this stage (E9.5), *Pax6* negatively regulates proliferation by repressing regulators of cell cycle progression (eg $p21^{cip1}$, $p27^{kip1}$, $p57^{Kip2}$) thus switching the focus towards progression and differentiation of the developing eye structure (Duparc *et al.*, 2007). Accordingly, $Pax6^{-/-}$ mice at this time exhibit overproliferation of optic vesicle precursor cells (Duparc *et al.*, 2007).

At later stages of eye development (E13.5), conditional gene targeting of *Pax6* demonstrated its ability to activate neuronal-specific genes such as *Math5*, *Mash1* and *Ngn2* at appropriate times, culminating in precise specification of multiple neuronal subtypes (Marquardt *et al.*,

2001). Accordingly, increasing or decreasing *Pax6* expression during early *Xenopus* eye field development increases or decreases retinal stem cell proliferation, respectively, and changes the differentiation profile of the retinal subtypes. However, the effect of altered Pax6 levels on proliferation is weakened in the mature retina, reflecting a functional switch from proliferation in early stages towards differentiation at later stages (Zaghloul and Moody, 2007). These results also implicate the involvement of distinct co-factors (both upstream and downstream) in the cellular response to *Pax* regulation.

This capacity of *Pax6* to invoke a temporally-sensitive switch from proliferation to differentiation within the retina parallels *Pax6* function elsewhere in the CNS. During cerebrocortical development, the absence of *Pax6* in conditional knockout mice results in overproliferation of early precursors and premature cell cycle exit (Estivill-Torrus *et al.*, 2002) with depletion of the progenitor pool available for late neurogenesis (Tuoc *et al.*, 2009). Conversely, overexpression of *Pax6* reduces proliferation of late cortical progenitors in a cell-autonomous and auto-regulated manner (Manuel *et al.*, 2007). Furthermore, Pax6-deficient embryonic stem cells transplanted into the dorsal telencephalon of the developing chick give rise to misspecified progenitors that generate GABAergic rather than glutamatergic neurons (Nikoletopoulou *et al.*, 2007).

Similarly, in adult neurogenesis, altered levels of *Pax6* in the rat hippocampus cause precocious progression of early progenitor cells to late stages (Maekawa *et al.*, 2005) or precocious differentiation into neuronal subclasses. This feature also exists within the developing spinal cord, where variable Pax6 levels are responsible for different functional outcomes; initially Pax6 promotes proliferation, then an increase in Pax6 within the cells of the ventricular zone invokes a switch determining cell cycle exit and cessation of

proliferation. Conversely, low levels of Pax6 favour maintenance of the progenitor state. In the developing spinal cord of *Pax6*$^{-/-}$ mice, loss of Pax6 leads to premature differentiation of neural precursor cells (Bel-Vialar *et al.*, 2007) and similarly causes precocious oligodendrogenesis and astrogenesis (Sugimori *et al.*, 2007). Under these circumstances, inappropriate neurons/glia may be formed due to differentiation in an incorrect environment and/or at the incorrect time (Hack *et al.*, 2005; Kohwi *et al.*, 2005; Philips *et al.*, 2005; Sugimori *et al.*, 2007), or there may be cell loss secondary to disrupting either the intricate balance between proliferation and differentiation (Kohwi *et al.*, 2005), or the relationship between the differentiating cell and its environment. An example of the latter occurs when precocious neurons formed in the rudimentary optic vesicle in *Pax6*$^{-/-}$ mice fail to persist (Philips *et al.*, 2005), indicating the pivotal relationship between the cell, the microenvironment, and the correct timing of differentiation.

Another illustrative example is the directed differentiation within the SVZ/olfactory bulb system whereby *Pax6* expression is maintained in a subset of adult SVZ progenitors which migrate to the olfactory bulb, where *Pax6* is downregulated and progenitors differentiate, producing the appropriate neuronal subclass. Although *Pax6*$^{-/-}$ progenitors transplanted into the SVZ of adult wildtype mice produce progenitor cells capable of correct migration, they undergo precocious differentiation and fail to generate particular subsets of neurons (Kohwi *et al.*, 2005). Interestingly, *Pax6* is not required for generation of dopaminergic periglomerular neurons during development (Mastick and Andrews, 2001), in contrast to its requisite role during their formation in adult neurogenesis (Brill *et al.*, 2008), providing another example of a highly complex cellular control based upon temporal variance.

So, differential regulation of/by *Pax6* provides the capacity for progenitor proliferation, maintenance, cell cycle progression and neurogenesis driven by variable Pax6 protein levels (Berger *et al*., 2007; Manuel *et al*., 2007; Sansom *et al.*, 2009; Tuoc *et al*., 2009). This capacity may also be affected by isoform variants, as the canonical (full-length) Pax6 protein regulates cell fate and proliferation, whilst the Pax6(5a) variant (binding of the PAI of the paired domain is abolished) regulates cell proliferation only during mouse CNS development (Haubst *et al.*, 2004), indicating distinct downstream targets for these functions. Similarly, the full-length Pax6 protein is present in the sub-ependymal zone and olfactory bulb, whereas the PD-less isoform (paired-less; lacks entire paired domain) is only present in the olfactory bulb, where it complexes with the full-length Pax6 protein to regulate neuronal survival via homeodomain-mediated DNA binding of crystallin-αA (Ninkovic *et al*., 2010). Thus a complex spatial and temporal Pax6 isoform profile is required during development for correct specification of neuronal subtypes.

The temporally-driven command of progenitor maintenance versus differentiation is also a recognised feature of other Pax proteins. Pax3, for example, at early stages maintains the undifferentiated phenotype of neural crest cells, but at later stages Pax3 binds directly to *cis*-regulatory elements in the promoter of *Ngn2* and thus may initiate differentiation of the neuronal lineage in the neural tube (Nakazaki *et al*., 2008). Within the ophthalmic trigeminal placode, *Pax3* activation is required for neuronal differentiation to occur; however, misexpression of *Pax3* in head ectoderm results in upregulation of proneural genes (eg *Ngn2*) without neuronal differentiation occurring, indicating a tissue-specific regulation for *Pax3* in neuronal differentiation (Dude *et al.*, 2009). This tissue-specific regulation is mediated perhaps by spatially restricted co-factors and/or by alternate isoforms (Charytonowicz *et al.*,

2011; Lamey *et al.*, 2004; Vogan and Gros, 1997; Ziman *et al.*, 1997; Ziman and Kay, 1998; Ziman *et al.*, 2001b).

To add further complexity, co-ordinated expression of multiple *Pax* genes may be required for correct development and definitive cell determination, such as the co-operative expression of *Pax6* and *Pax2* during CNS boundary formation. Another classic example of this co-ordinated expression occurs within the developing eye. At early optic vesicle stages, the co-ordinated and redundant activity of *Pax6* and *Pax2* specifies the retinal pigmented epithelia (Baumer *et al*., 2003). Divergent expression patterns at slightly later stages of optic cup morphogenesis determine the interface between the retina (*Pax6*-positive) and optic nerve (*Pax2*-positive), delineated by mutual *Pax6/Pax2* repression, thought to be achieved via the late retinal α-enhancer in the promoter of *Pax6*, which is repressed by Pax2 (Baumer *et al.*, 2002; Schwarz *et al.*, 2000). This mutual repression results in spatially and functionally distinct populations of cells (Schwarz *et al.*, 2000) - *Pax6*-positive retinal precursor cells (Marquardt *et al*., 2001) and *Pax2*-positive optic nerve astrocytes. Experimental inhibition of *Pax2* in embryonic mouse optic nerve explants causes upregulation of ectopic *Pax6* expression and ectopic neuronal differentiation (Soukkarieh *et al.*, 2007). Similarly, within the developing spinal cord *Pax2* maintains *Lhx1/Lhx5* and *Pax5/8* expression in dorsal horn interneurons for correct neuronal specification (Pillai *et al*., 2007). Within the ventral spinal cord, *Pax2* expression is initiated as cells become postmitotic and migrate laterally to the mantle zone. Preceding this, *Pax6* is required to initially specify these neural precursors prior to postmitotic emergence of neurons and does so by regulating expression of *Pax2* and other neuronal genes. Therefore, co-ordinated *Pax6* and *Pax2* expression co-operate to correctly specify ventral interneuron identity (Burrill *et al*., 1997).

Taken together, *Pax* genes maintain the undifferentiated cellular phenotype, and they participate in the timely decision to exit the cell cycle, and thus regulate differentiation to appropriate cell types based upon spatiotemporally permissive conditions, and, in some cases, co-operation between *Pax* family members and/or other co-factors. Identifying the mechanism underpinning the change in *Pax* function from proliferation to maintenance of the progenitor status to differentiation is a key challenge in deciphering *Pax* function from a regenerative perspective. It is likely to involve spatially regulated co-factors, as well as spatially regulated expression of alternate Pax isoforms, particularly those that involve modification of the C-terminus (Blake and Ziman, 2005; Charytonowicz *et al.*, 2011; Wang *et al.*, 2008; Wang *et al.*, 2007; Wang *et al.*, 2006) or regulation of the C-terminus by microRNAs (Chen *et al.*, 2010; Crist *et al.*, 2009; Dey *et al.*, 2011). These alternate isoforms may function differentially by regulating different downstream target genes (Charytonowicz *et al.*, 2011; Vogan and Gros, 1997; Wang *et al.*, 2007; Ziman *et al.*, 1997; Ziman and Kay, 1998; Ziman *et al.*, 2001a; Ziman *et al.*, 2001b). Thus, *Pax* genes are critical factors involved in progressing the spectrum of development from initial progenitor expansion and maintenance to correct neural differentiation (Bel-Vialar *et al.*, 2007; Berger *et al.*, 2007; Estivill-Torrus *et al.*, 2002; Lang *et al.*, 2005; Nakazaki *et al.*, 2008; Sansom *et al.*, 2009; Sugimori *et al.*, 2007). Collectively, these results also highlight an important feature of *Pax* genes - their ability to act as multipotent, spatiotemporally-programmed switches which are sensitive to environmental cues (Gerber *et al.*, 2002). It will be challenging to recapitulate this feature in the quest for “designer” cells for replacement purposes, and success will essentially rely on deciphering the genetic/epigenetic environmental factors involved in discriminating *Pax* function at different temporal and spatial levels of development.

9. POSTNATAL EXPRESSION PATTERNS OF *PAX* GENES

In addition to their well-accepted role in embryogenesis, the expression of *Pax* genes in adult regions is significant (Table 4), being required for maintenance of a progenitor cell phenotype (such as *Pax6* in adult neurogenesis) or for maintenance of plasticity in mature neurons in response to environmental stimuli (Gerber *et al.*, 2002). Conversely, the absence of *Pax6* in postnatal astrocytes reduces their neurogenic potential (Heins *et al.*, 2002). Additionally, Pax6 regulates survival of dopaminergic periglomerular neurons by inhibiting programmed cell death in these mature olfactory neurons (Ninkovic *et al.*, 2010).

Table 4: Postnatal expression patterns of *Pax* genes

***Pax* Gene**	**Adult animal cells showing *Pax* expression**	**References**
Pax2	GABAergic cerebellar interneurons (rabbit)	(Ponti *et al.*, 2008)
	Nuclei of the midbrain, pons/medulla and cerebellum (mouse)	(Stoykova and Gruss, 1994)
Pax6	Retinal cells, telencephalon, diencephalon, ventral mesencephalon, cerebellum and pons/medulla (various mammalian species)	(Nacher *et al.*, 2005; Stanescu *et al.*, 2007; Stoykova and Gruss, 1994)
	Neural progenitor cells of the SVZ/rostral migratory stream/olfactory bulb, the subgranular zone of the dentate gyrus of the hippocampus and the adult piriform complex	(Guo *et al.*, 2010; Hack *et al.*, 2005; Kohwi *et al.*, 2005; Maekawa *et al.*, 2005; Nacher *et al.*, 2005; Yamamoto *et al.*, 2001)
Pax3	Bergmann glia and cells surrounding Purkinje cells of the cerebellum (mouse)	(Stoykova and Gruss, 1994)
Pax7	Superior colliculus, specific nuclei of the pons/medulla and thalamus; cerebellar Bergmann glia (rat, mouse and chick)	(Shin *et al.*, 2003; Stoykova and Gruss, 1994; Thomas *et al.*, 2007; Thompson *et al.*, 2007; Thompson *et al.*, 2008)

10. *PAX* EXPRESSION SUBSEQUENT TO NEUROTRAUMA

One important aspect of neuroscience research is the quest for factors that influence the capacity for a cell to survive neurotrauma or neurodegeneration and/or the subsequent neuroinflammatory processes that ensue, and such discoveries will have a major impact on CNS cell therapy interventions. Whilst there is paucity of information regarding *Pax* genes in this regard, several studies have demonstrated the capacity for *Pax* genes to respond to neurotrauma and for cells expressing *Pax* to tolerate the post-insult environment.

Tonchev *et al* have demonstrated the capacity for newly-born *Pax6*-expressing neural progenitors to survive long term in both the subgranular zone (SGZ) of the hippocampal dentate gyrus (Tonchev and Yamashima, 2006) and the anterior SVZ (Tonchev *et al.*, 2006) after experimentally-induced transient global cerebral ischemia in primates, reinforcing that *Pax6*-expressing progenitors originating from the germinal zones are protected by *Pax6* expression. Similarly, *Pax*-expressing cells withstand injury in various tissues; *Pax6*- and *Pax7*-expressing cells remain within the injured adult rat spinal cord (Yamamoto *et al.*, 2001), whereas *Pax6* expression is upregulated in postnatal olfactory epithelium (Guo *et al.*, 2010) and re-expressed within retinal cells, including Müller glia (Bernardos *et al.*, 2007; Fischer and Reh, 2001; Hitchcock *et al.*, 1996; Karl *et al.*, 2008) after lesion.

Similarly, *Pax7* is re-expressed in adult rat superior collicular neurons after optic nerve transection (Thomas *et al.*, 2007); this may reflect an effect of reduced input. Moreover, increased numbers of *Pax7*-expressing cells were detected caudally after lesion to the rostral-medial superior colliculus, and expression remained elevated over a four week period (Thomas *et al.*, 2009). Taken together, these results indicate the capacity for *Pax*-expressant cells to survive environmental influences occurring post-insult. It is likely that survival

capacity may be differentially affected by distinct modes of injury. More work will be required to definitively assess the capacity of *Pax* genes to protect cells after trauma or in degenerating or inflammatory environments. Such knowledge will assist in delivering a functional, mature cell able to survive the transplanted environment after trauma or degeneration.

11. CURRENT STEM CELL THERAPY STRATEGIES FOR CNS REPAIR AND PAX GENES – GETTING IT RIGHT

Stem cell research is a dynamic area of investigation which harbours great promise for alleviation of neurological conditions. To date, transplant therapies have shown some success in patients and animal models of spinal cord injury (Amoh *et al.*, 2008; Hu *et al.*, 2010), stroke (Borlongan *et al.*, 1998; Hodges *et al.*, 1996; Sorensen *et al.*, 1996), Parkinson's disease (Falkenstein *et al.*, 2009; Kordower *et al.*, 1995; Thompson *et al.*, 2009), Huntington's disease (Capetian *et al.*, 2009; Deckel *et al.*, 1983; Freeman *et al.*, 2000) and retinal disorders (Radtke *et al.*, 2008; Radtke *et al.*, 2004). When used in these scenarios transplanted cells can survive (Hu et al., 2010), migrate (Bjugstad *et al.*, 2008; Wernig *et al.*, 2004), integrate (Bjugstad *et al.*, 2008; Borlongan *et al.*, 1998; Sorensen *et al.*, 1996) and produce some functional benefits (Deckel *et al.*, 1983; Isacson *et al.*, 1984; Pritzel *et al.*, 1986; Wictorin *et al.*, 1990). The use of fetal tissue transplants initially provided some promising results in patients with Parkinson's disease (Kordower *et al.*, 1995) and Huntington's disease (Bachoud-Levi *et al.*, 2000; Gaura *et al.*, 2004) but has been unfavourably impacted by treatment side effects such as dyskinesias in Parkinson's disease (Freed *et al.*, 2001; Greene *et al.*, 1999), and disease-like states occurring within the grafted cells, causing eventual graft degeneration in Huntington's disease (Cicchetti *et al.*, 2009) and, to a lesser extent, in Parkinson's disease (Kordower *et al.*, 2008a; Kordower *et al*., 2008b; Li *et al*., 2008).

It is evident that developmental genes involved in key cellular processes such as specification, proliferation, migration, differentiation and survival will be important mediators in directing stem cell therapy for CNS repair. As such, *Pax* genes are prime candidates for enhancement of future replacement strategies (Figure 3).

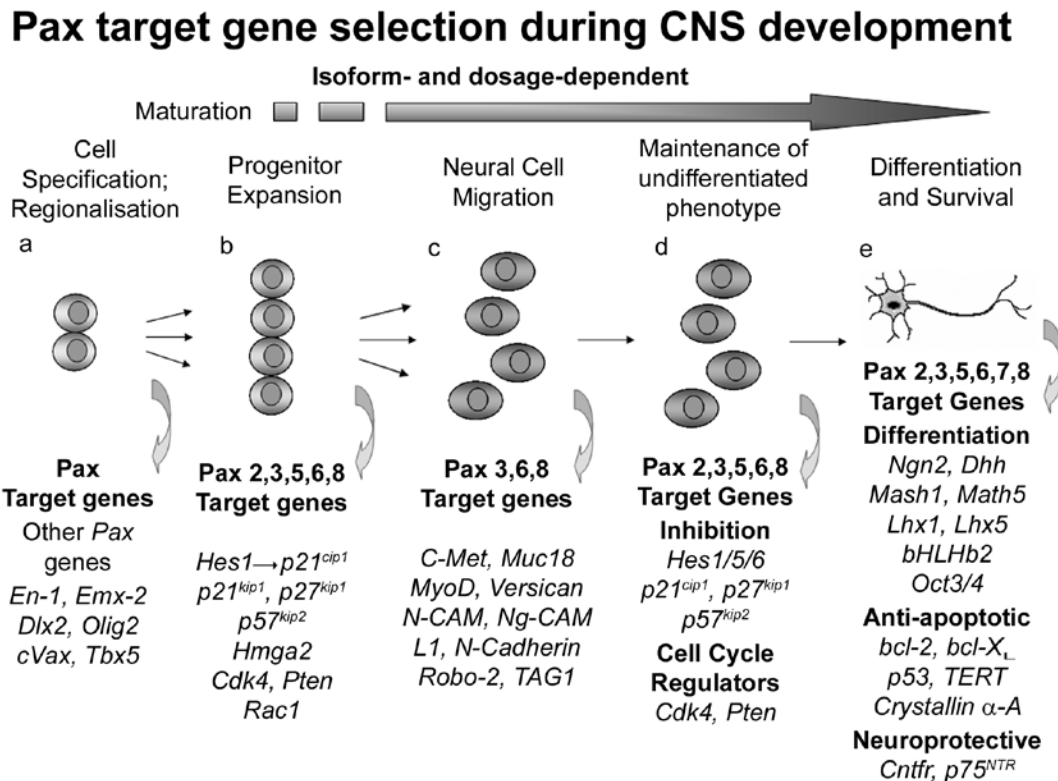

Figure 3 Thompson *Progress in Neurobiology*

<u>Figure 3</u>. Schematic of Pax function and downstream targets during CNS development, including cellular specification and regionalisation (a), progenitor expansion (b), neural cell migration (c), maintenance of the undifferentiated phenotype (d), differentiation, and maintenance of differentiated cells by cell survival and anti-apoptotic mechanisms (e). Listed target genes apply to one or more listed Pax proteins. Throughout these processes, Pax functions are concentration-dependent.

Due to very early neural expression and their capacity for neuronal specification, *Pax* genes may be powerful tools in directing differentiation pathways along desired routes, particularly

when manipulation of alternate isoforms or co-factors can be used to specify desired subtypes. In addition, *Pax* genes can specify progenitors that differentiate into radial glia, projection neurons, interneurons, astrocytes, oligodendrocytes and schwann cells (Chan-Ling *et al.*, 2009; Kioussi *et al.*, 1995; Mo and Zecevic, 2008; Pillai *et al.*, 2007; Sugimori *et al.*, 2007), and this may prove beneficial when use of heterogeneous populations may maximise repair and regeneration strategies. Furthermore, the ability to maintain a stem/progenitor cell phenotype and promote cellular survival provides a sound rationale for harnessing *Pax* genes for future stem cell therapies. These results also highlight the critical need for further work to reveal the identity and nature of other co-factors (eg epigenetic factors, upstream or downstream targets) that participate with *Pax* genes during these crucial developmental processes, and the functional peculiarity of isoforms to identify how, essentially, one transcription factor can multi-task and direct such diverse functional outcomes. More research detailing the capacity of *Pax* genes to supplement current repair strategies is likely to be beneficial.

Further considerations for future success of transplantation therapies will include the site of transplantation, the type of cell chosen and the transplant environment. The striatum has previously been chosen as the site of foetal graft transplantation in Parkinson's disease, however functional recovery is incomplete in human (Lindvall and Hagell, 2000) and animal models (Annett *et al*., 1994; Winkler *et al.*, 2000) and is thought due to ectopic placement of the grafts in an unfavourable microenvironment or lack of afferent input to grafted cells (Gaillard *et al*., 2009; Thompson *et al*., 2009). However, new research has indicated that foetal ventral mesencephalic cells transplanted into the 6-OHDA-lesioned adult mouse substantia nigra can integrate and restore the nigrostriatal pathway (Gaillard *et al*., 2009; Thompson *et al*., 2009) and this is enhanced with addition of appropriate neurotrophic

support (Thompson *et al*., 2009). Moreover, whilst foetal ventral mesencephalic cells transplanted into the substantia nigra produce dopaminergic cells capable of projecting to the striatum and restoring the nigrostriatal pathway, dopaminergic cells from the embryonic olfactory bulb do not (Gaillard *et al.*, 2009), indicating that intrinsic qualities of the cell impact its transplant capability and therefore matching transplanted cells with their environment may significantly influence transplant success.

Similarly, embryonic stem cells matured within tissue explants, allowing extrinsic signals within the tissue to direct maturation, appears favourable for the production of neural stem cells with the potential to recapitulate the dopaminergic development programme within the ventral mesencephalon. However, neuralization of these cells within explants produces more mature cells with a high neurogenic potential but low capacity to respond to environmental cues for site-specific differentiation, indicating that with progressive cellular maturation comes restricted plasticity (Baizabal and Covarrubias, 2009). This agrees with previous findings determining that in early stages of mesencephalic development, cells have a greater capacity to respond to extrinsic signals, and early progressive maturation is driven more by extrinsic (non-cell autonomous), rather than intrinsic (cell autonomous) cues. As cell maturation progresses, however, cells become more reliant on intrinsic qualities for specialisation of function (Li *et al*., 2005). Nevertheless, such use of an "instructive niche" may circumvent the need for modulating culture conditions to suit different spatiotemporal requirements (Baizabal and Covarrubias, 2009), as the plethora of contributing factors that must precisely intersect for correct cellular specification, as demonstrated in this review, is a daunting prospect. Taking these considerations into account, this protocol may provide an avenue to correctly ascribe *Pax* gene expression to improve transplant outcome when the

transplant environment (eg adult or post-traumatic) demonstrates poor efficacy to instruct the immature cell along a differentiation pathway.

Sadly, there has been scarcity in the recent literature detailing *Pax* gene use in manipulation of stem/progenitor cells for transplant therapies. Perhaps the complexity of the task has proved too intimidating. It is obvious from the research cited in this paper that *Pax* gene levels, alternate isoforms, co-factors and co-operation with paralogues (or other *Pax* genes) are required for correct structural and cellular determination. To mimic this level of precision within stem or progenitor cells before or after transplantation is a challenging task but appears plausible if the *Pax* master switch is provided in the right context. The ultimate question here is whether it is possible, using individual *Pax* genes, to recapitulate these processes and produce specific neurons from stem/progenitor cells within an *in vitro* situation. Given that *Pax* gene dosage is a critically sensitive variable in defining cell outcome, and the requirement for definitive upstream regulators of *Pax* genes (dynamic from a temporospatial perspective), it is tempting to speculate that use of a suitable "instructive niche" to generate and foster appropriate *Pax* expression levels prior to transplantation may provide a powerful mechanism to produce cells for neuro-restorative purposes. Further research in this area should provide exciting results.

Some success has been achieved where the capacity of *Pax* genes to specify neurons in embryonic or adult multipotent stem cells and enhance their proliferation and survival has been trialled for both endogenous and exogenous sources. In particular, *Pax6* has received noteworthy interest due to the capacity for cortical neurogenesis (Berger *et al.*, 2007; Estivill-Torrus *et al.*, 2002; Sansom *et al.*, 2009) and specification of dopaminergic neurons (Kohwi *et al.*, 2005) for neurodegenerative diseases such as Parkinson's disease. Recent experiments demonstrate that *Pax6* expression in embryonic stem cells directs neuroectoderm progression

toward a radial glial fate (neuronal precursors) (Suter *et al*., 2009). Moreover, use of *Pax6*-positive or *Pax6*-negative embryonic stem cells cultured in appropriate conditions prior to transplantation can give rise to glutamatergic or GABAergic cortical cells, respectively (Nikoletopoulou *et al*., 2007).

Whilst progression of neural stem cells *in vitro* toward a neuronal fate historically has been poor, *Pax6* overexpression in neurosphere cultures has been shown to direct neuronogenesis in almost all neurosphere-derived cells *in vitro* (Hack *et al*., 2004). Similarly, when Pax6 protein was delivered into E12 rat ventral mesencephalic neurosphere cultures, the neuronal progenitors increasingly adopted a dopaminergic fate (Spitere *et al*., 2008). In a transplant scenario, Kallur *et al* (2008) achieved increased generation of neuronal cells after transplanting *Pax6*-overexpressing human striatal neural stem cells into neonatal rat striatum.

It appears, however, that definitive cell lineage determination may be more specifically achieved by alternate isoforms. Pax7 isoforms can direct distinct lineages as suggested by varied expression patterns during development; myogenic-derived Pax7b induces neuronal differentiation in P19 embryonal carcinoma cells (Ziman *et al*., 1997; Ziman *et al.,* 2001b). Likewise, Pax6-5a isoform induces neuronal differentiation in murine embryonic stem cells *in vitro*, in contrast to the canonical Pax6 isoform, and it does so by regulating expression of *bHLHb2* and *Oct3/4* (Shimizu *et al*., 2009). Autoregulatory functions of Pax6 isoforms also stabilise relative levels of isoforms to achieve the desired outcome (Pinson *et al*., 2005; Pinson *et al*., 2006). These results collectively highlight the capacity for *Pax* genes to specify desired lineages for stem cell therapies, however knowledge of correct co-factors will be required.

As the use of human embryonic stem cells or foetal tissue for neuroregeneration is contentious due to ethical considerations and availability of tissue, identifying suitable cell types to circumvent this issue is crucial. Adult stem cells are potential candidates that have the added feature of autology, eliminating immunological rejection of the transplant. Adult stem cells may be harvested from an affected individual, re-specified (using *Pax* genes, for example) to produce the cell type of interest and transplanted back at the affected site. However, preparing cells in this manner for neurorepair will require fundamental knowledge of the key factors required to produce a "designer" cell of interest. For instance, bone marrow-derived adult human mesenchymal stem cells exhibit a predisposition for neural differentiation and express *Pax6 in vitro* under the appropriate conditions (Blondheim *et al.*, 2006) and hence achieve some functional repair when transplanted into various rat models of brain and spinal cord injury (Chen *et al.*, 2001; Chopp *et al.*, 2000; Li *et al.*, 2001; Lu *et al.*, 2001; Mahmood *et al.*, 2001).

In order to achieve functional improvements after transplantation, the chosen cell type should be compatible with the host brain region and must be capable of integrating into circuitry regulated by the host brain environment (Isacson, 2003). Achieving an optimum match between cell and target site may require manipulation of the cell and/or the environment, and may be augmented by the use of factors such as neurotrophins (Choi *et al.*, 2010; Thompson *et al.*, 2009; Yang *et al.*, 2010). In support of this, mesencephalic neuroepithelial stem cells grafted into damaged rat striata show increased survival and differentiation tendencies compared to grafts into intact striata, indicating the powerful influence of the environment on the cell (Mine *et al.*, 2009).

Interestingly, a variety of stem cell types have been used to repair the retina, albeit with differing levels of success. Ciliary retinal stem cells from the adult human eye (Pax6-positive) have shown some success in integrating and differentiating into photoreceptors and retinal pigmented epithelia post-transplant in postnatal NOD/SCID mice and embryonic chick retinae (Coles *et al.*, 2004), whereas some studies have shown that neural stem cells fail to fully differentiate into retinal phenotypes (reviewed in Baker and Brown, 2009), highlighting differences in transplant response which may be due to the potency of the cells chosen and their more closely-matched compatibility with the environment. A recent study assessed the capacity for *Pax7* to enhance CNS repair by matching the transcription profile of donor cells to that of the host tissue. *Pax7*-expressing neural progenitor cells taken from embryonic rat dorsal mesencephalon were grafted within the adult rat dorsal mesencephalon (Pax7-positive) or ventral mesencephalon (Pax7-negative), and whilst overall graft survival did not vary, the number of resultant astrocytes was reduced when *Pax7*-expressing cells were grafted into a non-*Pax7*-expressing region (Thomas *et al.*, 2009). These experiments also highlight the capacity of *Pax*-expressant cells to withstand inflammation and trauma (Edwards *et al.*, 1986a; Finlay *et al.*, 1982), possibly due to transcriptional regulation of survival factors (reviewed in Medic and Ziman, 2009; Ninkovic *et al.*, 2010; White and Ziman, 2008) - an important feature that will significantly assist in neuroregenerative strategies.

It is apparent then that successful cell replacement requires knowledge of the appropriate cell type and maturation stage (Denham *et al.*, 2010). Pre-differentiation of cells *in vitro* into the appropriate cell type/s and maturation stage prior to transplantation has been attempted with variable success (Baizabal and Covarrubias, 2009; Le Belle *et al.*, 2004; Park *et al.*, 2009), possibly due to other factors inhibiting the *in vivo* uptake, integration and survival of mature transplanted cells within the injured environment. Another possible source of cells for

transplantation is via the targeted de-differentiation of mature cells, such as the use of pigment cells de-differentiated in culture conditions to produce neural crest-derived ancestor cells (Real *et al*., 2006). Correspondingly, forced expression of *Pax6* in postnatal cortical astroglia can instruct neurogenesis (Berninger *et al.*, 2007; Heins *et al.*, 2002), and under appropriate culture conditions, *Pax6* transfection into mouse embryonic stem cells results in cell-fate adaptation to corneal epithelial-like cells (Ueno *et al*., 2007). Similarly, *Pax6* can affect adult multipotent stem cell lineage specification; *Pax6* upregulation results in trans-differentiation of hair follicle stem cells into corneal epithelial-like cells in conditioned media (Yang *et al.*, 2009) or retinal pigmented epithelia into neuroretina in chick and *Xenopus* embryos (Arresta *et al*., 2005; Azuma *et al*., 2005).

Environmental factors subsequent to inflammation and injury also significantly influence neuroregenerative therapies. In Huntington's disease, inherent immunological functions may cause degeneration of striatal grafted cells, which show differential survival rates in the caudate compared to the putamen (Cicchetti *et al*., 2009). Similarly, in Parkinson's disease Lewy bodies may eventually form in grafted cells (Kordower *et al.*, 2008a; Kordower *et al.*, 2008b; Li *et al*., 2008). However, whilst neuroinflammatory processes have generally been considered a negative component of CNS repair, evidence is emerging that chemokines and cytokines of the early immune response, involved in attracting inflammatory cells, also attract stem cells to the area of injury (Imitola *et al.*, 2004; Newman *et al.*, 2005). Therefore, injury-induced factors may positively affect transplant success, as demonstrated by the capacity of retinal stem cells to incorporate into the lesioned rat retina (Chacko *et al.*, 2003), and likewise the migration of neural stem cells to infarcted areas due to mediators of the inflammatory response (Imitola *et al*., 2004). Furthermore, the inability of pre-differentiated neurons and the capacity of stem cells to migrate to injury sites indicates a certain level of plasticity is

required for correct migration to pathological sites, or that differentiated cells respond differently to migration/survival cues (Park *et al.*, 2009), and this will impact the maturity level of cells chosen or definitive Pax isoform selected for different applications, as cell migration after transplant is not always a desirable characteristic.

Therefore, the use of transcription factors to "prime" cells by matching the genetic profile of transplanted cells to the damaged environment may optimise transplant success (Kallur *et al.*, 2008; Thomas *et al.,* 2009). Moreover, conditions that manipulate this dictated gene expression and the cell type chosen to exploit it, as well as the ability to manipulate the environment for graft uptake, will depend upon the nature of the condition being assessed. Thus to successfully manipulate cells to survive, integrate and mature to produce significant functional restoration to circuitry and information processing after *in vitro* conditioning requires investigation specific for each condition (Baizabal and Covarrubias, 2009; Srivastava *et al.*, 2008). Additionally, the appropriate use of stem cell survival factors (including *Pax*) to assist with transplant survival (Pluchino *et al.*, 2010; Sieber-Blum, 2010) may be utilised to improve graft outcomes.

12. FURTHER CONSIDERATIONS

Whilst it is clear that Pax transcription factors possess many promising features that offer substantial promise for CNS regenerative strategies, their anti-apoptotic and oncogenic potential, as detailed below, will require further consideration when utilising *Pax* genes for neuroregenerative purposes.

Correct embryo formation is also critically achieved by regulating apoptosis to create the optimal number of cells and/or architecture of the developing tissue, particularly in the CNS.

Pax genes mediate cell survival by inhibiting apoptosis in many regions of the body to direct organogenesis or for maintenance of normal homeostatic mechanisms. In *Pax2*$^{-/-}$ mice, optic stalk cells degenerate (Schwarz *et al.*, 2000), and *Pax2*$^{+/-}$ mice exhibit renal-coloboma syndrome. When a *Pax5* minigene is inserted into the *Pax2* locus, most functionality is restored due to redundancy, however symptoms similar to *Pax2*$^{+/-}$ remain in the kidney and eye. Whilst cell proliferation is normal, there is increased apoptosis (Bouchard *et al.*, 2000), revealing a dosage-dependent, anti-apoptotic role for *Pax2*. Within the mouse kidney, *Pax2* protects against osmotic-induced apoptosis (Cai *et al.*, 2005) by indirectly regulating the anti-apoptotic gene *bcl-2* via the transcription factor WT1 (Bouchard *et al.*, 2000), similar to the indirect regulation of *bcl-*X_L by *Pax5* during B-lymphopoiesis (Nutt *et al.*, 1998). Direct modulation of *bcl-2* (homologue *ced-9*) by *Pax2/5/8* genes has been demonstrated in *C.elegans* (Park *et al.,* 2006). Moreover, *Pax2/5/8* expression is inversely correlated with expression of the tumour suppressor gene *p53* in astrocytoma and directly inhibits activity of the *p53* promoter *in vitro* (Stuart *et al*., 1995a; Stuart *et al*., 1995b). *Pax8* also activates the anti-apoptotic *TERT* (*Telomerase Reverse Transcriptase*) gene in glioma cell lines, implicating it in glioma cell survival (Chen *et al.*, 2008).

An anti-apoptotic role for *Pax3* is demonstrated by *Pax3* inhibition secondary to maternal diabetes (Phelan *et al*., 1997), whereby neuroepithelial cells undergo apoptosis via *p53*-dependent mechanisms (Pani *et al.*, 2002), explaining neural tube defects induced in diabetic pregnancy. This was demonstrated by the rescue of anti-apoptotic function in *Pax3*$^{-/-}$ mice with *p53* loss-of-function (Pani *et al*., 2002), and by the observation that *Pax3* inhibits *p53* activity *in vitro* by modulating its transcriptional activity and by promoting degradation of the p53 protein (Underwood *et al*., 2007).

Evidence for an anti-apoptotic function of *Pax6* was recently demonstrated whereby Pax6 regulated survival of dopaminergic OB neurons during adult neurogenesis via direct regulation of crystallin-αA, which prevents activation of the caspase cascade and thus inhibits programmed cell death (Ninkovic *et al.*, 2010). Pax6 also negatively regulates expression of the neurotrophic receptor $p75^{NTR}$ (Nikoletopoulou *et al.,* 2007), demonstrated to cause neuronal death when overexpressed (Majdan *et al.*, 1997; Plachta *et al*., 2007). Therefore, *Pax6* mutant mice exhibit premature neuronal differentiation accompanied by rapid cell death of mis-specified neurons (Nikoletopoulou *et al*., 2007).

Thus it appears that *Pax* genes couple early stages of neural development (specification/maintenance) to later stages (differentiation/maintenance) by providing anti-apoptotic mechanisms throughout these processes, and this may also be differentially achieved using alternate isoforms and their subsequent ability to discriminate distinct downstream targets (Wang *et al.*, 2007; Wang *et al.*, 2006; Zhang *et al.*, 2010). Indeed, investigation of Pax3 isoforms stably transfected into mouse melanocytes *in vitro* has demonstrated differing isoform-specific effects on cell function, which is achieved by differential regulation of distinct downstream targets, including genes involved in proliferation and survival (*Rac1*), differentiation (*Dhh*), transcriptional repression of *Pax3* (*Msx1*) and migration/transformation (*Met, Muc18*) (Wang *et al.*, 2007; Wang *et al.*, 2006). Whilst this feature is advantageous in the context of normal developmental processes, it may have deleterious consequences from an oncogenic perspective in putative regeneration strategies.

Whilst not a focus of this review, a discussion of *Pax* gene function in cell replacement therapy would not be complete without due regard to their oncogenic potential (refer Wang *et*

al., 2008 for comprehensive review), due to the risk of instigating tumourigenesis when using non-terminally differentiated cells (Heine *et al*., 2004; Johnson *et al.*, 2008). *Pax* genes are implicated in a wide variety of cancers, presumably due to regulation of proliferation, cell cycle arrest, migration and cell survival, and it has been proposed that different *Pax* groups or different Pax isoforms may pose a greater cancer risk due to structural and functional variation (Robson *et al.*, 2006; Wang *et al.*, 2008; Wang *et al.*, 2007; Wang *et al.*, 2006).

Pax8 is overexpressed in glioma (Tong *et al*., 2008), and *Pax3* and *Pax7* are expressed in a variety of neuroectodermal tumours (Gershon *et al*., 2005). However, whilst *Pax5* deregulation and overexpression have been reported in medulloblastoma (Kozmik *et al*., 1995) and expression noted in astrocytoma (Stuart *et al*., 1995b), manipulation forcing overexpression in an effort to induce brain tumour formation from mouse neuroectoderm was unsuccessful (Steinbach *et al.*, 2001), suggesting caution when inferring causality from expression patterns. Conversely, the association of *Pax5* with haematopoietic cancers such as B-Cell lymphoma (Busslinger *et al*., 1996) and acute lymphoblastic leukaemia (Nebral *et al*., 2007), together with small cell-lung cancer (Kanteti *et al*., 2009) suggests tissue-specific oncogenic capabilities (Steinbach *et al*., 2001). Knockdown of *Pax2* (ovarian/bladder) or *Pax3* (melanoma) in cancer cell lines (Muratovska *et al.*, 2003), and *Pax3* and *Pax7* in alveolar rhabdomyosarcoma cells (Bernasconi *et al.,* 1996), results in rapidly induced apoptosis, with a demonstrated anti-apoptotic pathway being the negative association between *Pax* genes and *p53* (Stuart *et al*., 1995a; Underwood *et al.*, 2007). Collectively, these data suggest that *Pax* genes may bestow a cell survival mechanism on cancer cells, protecting them from normal elimination processes. This risk may, however, be reduced with careful choice of the appropriate Pax isoform.

13. CONCLUSION

As demonstrated in this review, *Pax* genes participate in almost all facets of CNS development, from the earliest to mature stages. Whilst their function in mature, differentiated adult cells still proves enigmatic, there is a wealth of evidence identifying complex and important roles for *Pax* genes in orchestrating and co-ordinating multiple aspects of neural maturation.

Initially, *Pax* genes dictate correct organogenesis by ensuring sufficient progenitor cells for organ development. This will impact stem cell therapies by ensuring initial expansion of cells if culture conditions can recapitulate this *in vitro*. Secondly, the capacity for *Pax* genes to maintain the undifferentiated status of the cell until directed to switch towards differentiation allows for a variable, spatiotemporal-driven specification capable of producing different mature cell types within a changing developmental niche. This demonstrates their aptitude as multipotent switches, instructing cells along differential pathways depending on the cell history and its spatial placement (Torres *et al.*, 1996), providing credence for the previously suggested paradigm of *Pax* function; the capacity to couple extrinsic (environmental) and intrinsic (cellular) signals by rendering the *Pax*-expressant cell responsive to spatiotemporal environmental cues (Blake *et al.*, 2008). This feature affords a powerful tool for stem cell therapy, provided the appropriate *Pax* expression can be partnered with a correctly instructed and permissive environment.

The challenge in harnessing *Pax* genes for stem cell therapy will not only lie in matching *Pax* and environment, but also in producing a cell with a correct complement of *Pax* dosage, including relative isoform levels, that is compatible with the environment at that point in time and place to achieve the desired outcome. The *Pax* family of genes display crucial, dosage-

dependent mechanisms for many functions (Hill *et al.*, 1991; Kanakubo *et al.*, 2006; Maekawa *et al.*, 2005; Thompson *et al.*, 2008; Zhou *et al.*, 2008), and overexpression has been implicated in tumourigenesis. For this reason, it is questionable whether transfection techniques can correctly assign *Pax* expression to a cell from a dosage perspective, and it may be more efficacious to use the "instructive niche" concept (Baizabal and Covarrubias, 2009) utilising the appropriate environment for onset of *Pax* expression.

Importantly, the ability of *Pax* genes to specify multiple cell lineages may have significant applications for therapeutic interventions requiring multiple cellular phenotypes. It will be crucial to understand which *Pax* isoforms and downstream targets facilitate cell fate choice as this can be exploited to direct differentiation to desired populations as required. Furthermore, the perceived capacity for *Pax* genes to respond to injury or stress suggests that either *Pax* genes may recapitulate the embryonic state for regenerative purposes, or highlights their roles as pro-survival/anti-apoptotic mediators. It is clear that neuroinflammatory processes themselves greatly influence remedial therapy, and stem cell therapies must be able to withstand these processes. It will therefore be necessary to identify what (if any) regeneration signals *Pax* genes respond to in the CNS and how these *Pax*-expressant cells interact with a damaged or regenerating environment. This will provide further insight into the applicability of *Pax* genes for CNS repair.

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REFERENCES

Amariglio, N., Hirshberg, A., Scheithauer, B. W., Cohen, Y., Loewenthal, R., Trakhtenbrot, L., Paz, N., Koren-Michowitz, M., Waldman, D., Leider-Trejo, L., Toren, A., Constantini, S. and Rechavi, G., 2009. Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. PLoS Med 6, e1000029.

Amoh, Y., Li, L., Katsuoka, K. and Hoffman, R. M., 2008. Multipotent hair follicle stem cells promote repair of spinal cord injury and recovery of walking function. Cell Cycle 7, 1865-1869.

Annett, L. E., Martel, F. L., Rogers, D. C., Ridley, R. M., Baker, H. F. and Dunnett, S. B., 1994. Behavioral assessment of the effects of embryonic nigral grafts in marmosets with unilateral 6-OHDA lesions of the nigrostriatal pathway. Exp. Neurol. 125, 228-246.

Apuzzo, S. and Gros, P., 2007. Cooperative interactions between the two DNA binding domains of Pax3: helix 2 of the paired domain is in the proximity of the amino terminus of the homeodomain. Biochemistry (Mosc). 46, 2984-2993.

Arresta, E., Bernardini, S., Bernardini, E., Filoni, S. and Cannata, S. M., 2005. Pigmented epithelium to retinal transdifferentiation and Pax6 expression in larval Xenopus laevis. J Exp Zoolog A Comp Exp Biol 303, 958-967.

Auerbach, R., 1954. Analysis of the developmental effects of a lethal mutation in the house mouse. J. Exp. Zool. 127, 305-329.

Azuma, N., Tadokoro, K., Asaka, A., Yamada, M., Yamaguchi, Y., Handa, H., Matsushima, S., Watanabe, T., Kida, Y., Ogura, T., Torii, M., Shimamura, K. and Nakafuku, M., 2005. Transdifferentiation of the retinal pigment epithelia to the neural retina by transfer of the Pax6 transcriptional factor. Hum. Mol. Genet. 14, 1059-1068.

Bachoud-Levi, A. C., Remy, P., Nguyen, J. P., Brugieres, P., Lefaucheur, J. P., Bourdet, C., Baudic, S., Gaura, V., Maison, P., Haddad, B., Boisse, M. F., Grandmougin, T., Jeny, R., Bartolomeo, P., Dalla Barba, G., Degos, J. D., Lisovoski, F., Ergis, A. M., Pailhous, E., Cesaro, P., Hantraye, P. and Peschanski, M., 2000. Motor and cognitive improvements in patients with Huntington's disease after neural transplantation. Lancet 356, 1975-1979.

Baizabal, J. M. and Covarrubias, L., 2009. The embryonic midbrain directs neuronal specification of embryonic stem cells at early stages of differentiation. Dev. Biol. 325, 49-59.

Baker, P. S. and Brown, G. C., 2009. Stem-cell therapy in retinal disease. Curr. Opin. Ophthalmol. 20, 175-181.

Balczarek, K. A., Lai, Z.-C. and Kumar, S., 1997. Evolution and functional diversification of the Paired Box (Pax) DNA-binding domains. Mol. Biol. Evol. 14, 829-842.

Bang, A. G., Papalopulu, N., Kintner, C. and Goulding, M. D., 1997. Expression of Pax-3 is initiated in the early neural plate by posteriorizing signals produced by the organizer and by posterior non-axial mesoderm. Development 124, 2075-2085.

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.

Baumer, N., Marquardt, T., Stoykova, A., Ashery-Padan, R., Chowdhury, K. and Gruss, P., 2002. Pax6 is required for establishing naso-temporal and dorsal characteristics of the optic vesicle. Development 129, 4535-4545.

Baumer, N., Marquardt, T., Stoykova, A., Spieler, D., Treichel, D., Ashery-Padan, R. and Gruss, P., 2003. Retinal pigmented epithelium determination requires the redundant activities of Pax2 and Pax6. Development 130, 2903-2915.

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.

Berger, J., Berger, S., Tuoc, T. C., D'Amelio, M., Cecconi, F., Gorski, J. A., Jones, K. R., Gruss, P. and Stoykova, A., 2007. Conditional activation of Pax6 in the developing cortex of transgenic mice causes progenitor apoptosis. Development 134, 1311-1322.

Bernardos, R. L., Barthel, L. K., Meyers, J. R. and Raymond, P. A., 2007. Late-stage neuronal progenitors in the retina are radial Muller glia that function as retinal stem cells. J. Neurosci. 27, 7028-7040.

Bernasconi, M., Remppis, A., Fredericks, W. J., Rauscher, F. J. r. and Schafer, B. W., 1996. Induction of apoptosis in rhabdomyosarcoma cells through down-regulation of PAX proteins. Proc. Natl. Acad. Sci. U. S. A. 93, 13164-13169.

Berninger, B., Costa, M. R., Koch, U., Schroeder, T., Sutor, B., Grothe, B. and Gotz, M., 2007. Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. J. Neurosci. 27, 8654-8664.

Betters, E., Liu, Y., Kjaeldgaard, A., Sundstrom, E. and Garcia-Castro, M. I., 2010. Analysis of early human neural crest development. Dev. Biol. 344, 578-592.

Bishop, K. M., Goudreau, G. and O'Leary, D. D. M., 2000. Regulation of area identity in the mammalian neocortex by *Emx2* and *Pax6*. Science 288, 344-349.

Bjugstad, K. B., Teng, Y. D., Redmond, D. E. J., Elsworth, J. D., Roth, R. H., Cornelius, S. K., Snyder, E. Y. and Sladek, J. R. J., 2008. Human neural stem cells migrate along the nigrostriatal pathway in a primate model of Parkinson's disease. Exp. Neurol. 211, 362-369.

Blake, J. A., Thomas, M., Thompson, J. A., White, R. and Ziman, M., 2008. Perplexing Pax: from puzzle to paradigm. Dev. Dyn. 237, 2791-2803.

Blake, J. A. and Ziman, M. R., 2005. Pax3 transcripts in melanoblast development. Dev. Growth Differ. 47, 627-635.

Blondheim, N. R., Levy, Y. S., Ben-Zur, T., Burshtein, A., Cherlow, T., Kan, I., Barzilai, R., Bahat-Stromza, M., Barhum, Y., Bulvik, S., Melamed, E. and Offen, D., 2006. Human mesenchymal stem cells express neural genes, suggesting a neural predisposition. Stem Cells Dev 15, 141-164.

Borlongan, C. V., Tajima, Y., Trojanowski, J. Q., Lee, V. M. and Sanberg, P. R., 1998. Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2N cells) promotes functional recovery in ischemic rats. Exp. Neurol. 149, 310-321.

Bouchard, M., Pfeffer, P. and Busslinger, M., 2000. Functional equivalence of the transcription factors Pax2 and Pax5 in mouse development. Development 127, 3703-3713.

Brill, M. S., Snapyan, M., Wohlfrom, H., Ninkovic, J., Jawerka, M., Mastick, G. S., Ashery-Padan, R., Saghatelyan, A., Berninger, B. and Gotz, M., 2008. A dlx2- and pax6-dependent transcriptional code for periglomerular neuron specification in the adult olfactory bulb. J. Neurosci. 28, 6439-6452.

Brun, T., Franklin, I., St-Onge, L., Biason-Lauber, A., Schoenle, E. J., Wollheim, C. B. and Gauthier, B. R., 2004. The diabetes-linked transcription factor PAX4 promotes {beta}-cell proliferation and survival in rat and human islets. J. Cell Biol. 167, 1123-1135.

Burrill, J. D., Moran, L., Goulding, M. D. and Saueressig, H., 1997. PAX2 is expressed in multiple spinal cord interneurons, including a population of EN1+ interneurons that require PAX6 for their development. Development 124, 4493-4503.

Busslinger, M., Klix, N., Pfeffer, P., Graninger, P. G. and Kozmik, Z., 1996. Deregulation of PAX-5 by translocation of the Emu enhancer of the IgH locus adjacent to two alternative PAX-5 promoters in a diffuse large-cell lymphoma. Proc. Natl. Acad. Sci. U. S. A. 93, 6129-6134.

Cai, Q., Dmitrieva, N. I., Ferraris, J. D., Brooks, H. L., van Balkom, B. W. and Burg, M., 2005. Pax2 expression occurs in renal medullary epithelial cells in vivo and in cell culture, is osmoregulated, and promotes osmotic tolerance. Proc. Natl. Acad. Sci. U. S. A. 102, 503-508.

Callaerts, P., Halder, G. and Gehring, W. J., 1997. PAX-6 in development and evolution. Annu. Rev. Neurosci. 20, 483-532.

Cao, Q., Xu, X. M., Devries, W. H., Enzmann, G. U., Ping, P., Tsoulfas, P., Wood, P. M., Bunge, M. B. and Whittemore, S. R., 2005. Functional recovery in traumatic spinal cord injury after

transplantation of multineurotrophin-expressing glial-restricted precursor cells. J. Neurosci. 25, 6947-6957.

Capetian, P., Knoth, R., Maciaczyk, J., Pantazis, G., Ditter, M., Bokla, L., Landwehrmeyer, G. B., Volk, B. and Nikkhah, G., 2009. Histological findings on fetal striatal grafts in a Huntington's disease patient early after transplantation. Neuroscience 160, 661-675.

Caric, D., Gooday, D., Hill, R. E., McConnell, S. K. and Price, D. J., 1997. Determination of the migratory capacity of embryonic cortical cells lacking the transcription factor Pax-6. Development 124, 5087-5096.

Chacko, D. M., Das, A. V., Zhao, X., James, J., Bhattacharya, S. and Ahmad, I., 2003. Transplantation of ocular stem cells: the role of injury in incorporation and differentiation of grafted cells in the retina. Vision Res. 43, 937-946.

Chan-Ling, T., Chu, Y., Baxter, L., Weible Ii, M. and Hughes, S., 2009. In vivo characterization of astrocyte precursor cells (APCs) and astrocytes in developing rat retinae: differentiation, proliferation, and apoptosis. Glia 57, 39-53.

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.

Chen, J., Li, Y., Wang, L., Lu, M., Zhang, X. and Chopp, M., 2001. Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. J. Neurol. Sci. 189, 49-57.

Chen, J. F., Tao, Y., Li, J., Deng, Z., Yan, Z., Xiao, X. and Wang, D. Z., 2010. microRNA-1 and microRNA-206 regulate skeletal muscle satellite cell proliferation and differentiation by repressing Pax7. J. Cell Biol. 190, 867-879.

Chen, Y. J., Campbell, H. G., Wiles, A. K., Eccles, M. R., Reddel, R. R., Braithwaite, A. W. and Royds, J. A., 2008. PAX8 regulates telomerase reverse transcriptase and telomerase RNA component in glioma. Cancer Res. 68, 5724-5732.

Choi, Y. J., Li, W. Y., Moon, G. J., Lee, P. H., Ahn, Y. H., Lee, G. and Bang, O. Y., 2010. Enhancing trophic support of mesenchymal stem cells by ex vivo treatment with trophic factors. J. Neurol. Sci. 298, 28-34.

Chopp, M., Zhang, X. H., Li, Y., Wang, L., Chen, J., Lu, D., Lu, M. and Rosenblum, M., 2000. Spinal cord injury in rat: treatment with bone marrow stromal cell transplantation. Neuroreport 11, 3001-3005.

Christophorou, N. A., Mende, M., Lleras-Forero, L., Grocott, T. and Streit, A., 2010. Pax2 coordinates epithelial morphogenesis and cell fate in the inner ear. Dev. Biol. 345, 180-190.

Cicchetti, F., Saporta, S., Hauser, R. A., Parent, M., Saint-Pierre, M., Sanberg, P. R., Li, X. J., Parker, J. R., Chu, Y., Mufson, E. J., Kordower, J. H. and Freeman, T. B., 2009. Neural transplants in patients with Huntington's disease undergo disease-like neuronal degeneration. Proc. Natl. Acad. Sci. U. S. A. 106, 12483-12488.

Coles, B. L., Angenieux, B., Inoue, T., Del Rio-Tsonis, K., Spence, J. R., McInnes, R. R., Arsenijevic, Y. and van der Kooy, D., 2004. Facile isolation and the characterization of human retinal stem cells. Proc. Natl. Acad. Sci. U. S. A. 101, 15772-15777.

Collombat, P., Xu, X., Ravassard, P., Sosa-Pineda, B., Dussaud, S., Billestrup, N., Madsen, O. D., Serup, P., Heimberg, H. and Mansouri, A., 2009. The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into alpha and subsequently beta cells. Cell 138, 449-462.

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.

Crist, C. G., Montarras, D., Pallafacchina, G., Rocancourt, D., Cumano, A., Conway, S. J. and Buckingham, M., 2009. Muscle stem cell behavior is modified by microRNA-27 regulation of Pax3 expression. Proc. Natl. Acad. Sci. U. S. A. 106, 13383-13387.

Crossley, P. H., Martinez, S. and Martin, G. R., 1996. Midbrain development induced by Fgf8 in the chick embryo. Nature 380, 66-68.

Dahl, E., Koseki, H. and Balling, R., 1997. Pax genes and organogenesis. Bioessays 19, 755-765.

Deckel, A. W., Robinson, R. G., Coyle, J. T. and Sanberg, P. R., 1983. Reversal of long-term locomotor abnormalities in the kainic acid model of Huntington's disease by day 18 fetal striatal implants. Eur. J. Pharmacol. 93, 287-288.

Denham, M., Thompson, L. H., Leung, J., Pebay, A., Bjorklund, A. and Dottori, M., 2010. Gli1 is an inducing factor in generating floor plate progenitor cells from human embryonic stem cells. Stem Cells 28, 1805-1815.

Dey, B. K., Gagan, J. and Dutta, A., 2011. miR-206 and -486 induce myoblast differentiation by downregulating Pax7. Mol. Cell. Biol. 31, 203-214.

Dude, C. M., Kuan, C. Y., Bradshaw, J. R., Greene, N. D., Relaix, F., Stark, M. R. and Baker, C. V., 2009. Activation of Pax3 target genes is necessary but not sufficient for neurogenesis in the ophthalmic trigeminal placode. Dev. Biol. 326, 314-326.

Duparc, R. H., Abdouh, M., David, J., Lepine, M., Tetreault, N. and Bernier, G., 2007. Pax6 controls the proliferation rate of neuroepithelial progenitors from the mouse optic vesicle. Dev. Biol. 301, 374-387.

Edelman, G. M. and Jones, F. S., 1998. Gene regulation of cell adhesion: a key step in neural morphogenesis. Brain Res. Brain Res. Rev. 26, 337-352.

Edwards, M. A., Caviness, V. S. J. and Schneider, G. E., 1986a. Development of cell and fiber lamination in the mouse superior colliculus. J. Comp. Neurol. 248, 395-409.

Epstein, J. A., Shapiro, D. N., Cheng, J., Lam, P. Y. and Maas, R. L., 1996. Pax3 modulates expression of the c-Met receptor during limb muscle development. Proc. Natl. Acad. Sci. U. S. A. 93, 4213-4218.

Erdo, F., Buhrle, C., Blunk, J., Hoehn, M., Xia, Y., Fleischmann, B., Focking, M., Kustermann, E., Kolossov, E., Hescheler, J., Hossmann, K. A. and Trapp, T., 2003. Host-dependent tumorigenesis of embryonic stem cell transplantation in experimental stroke. J. Cereb. Blood Flow Metab. 23, 780-785.

Ericson, J., Morton, S., Kawakami, A. and Fujisawa, H., 1996. Two critical periods of sonic hedgehog signalling required for the specification of motor neuron identity. Cell 87, 661-673.

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.

Estivill-Torrus, G., Pearson, H., van Heyningen, V., Price, D. J. and Rashbass, P., 2002. Pax6 is required to regulate the cell cycle and the rate of progression from symmetrical to asymmetrical division in mammalian cortical progenitors. Development 129, 455-466.

Falkenstein, G., Rosenthal, C., Reum, T., Morgenstern, R., Dobrossy, M. and Nikkhah, G., 2009. Pattern of long-term sensorimotor recovery following intrastriatal and--accumbens DA micrografts in a rat model of Parkinson's disease. J. Comp. Neurol. 515, 41-55.

Fedtsova, N., Quina, L. A., Wang, S. and Turner, E. E., 2008. Regulation of the development of tectal neurons and their projections by transcription factors Brn3a and Pax7. Dev. Biol. 316, 6-20.

Finlay, B. L., Berg, A. T. and Sengelaub, D. R., 1982. Cell death in the mammalian visual system during normal development: II. Superior colliculus. J. Comp. Neurol. 204, 318-324.

Fischer, A. J. and Reh, T. A., 2001. Muller glia are a potential source of neural regeneration in the postnatal chicken retina. Nat. Neurosci. 4, 247-252.

Fogel, J. L., Chiang, C., Huang, X. and Agarwala, S., 2008. Ventral specification and perturbed boundary formation in the mouse midbrain in the absence of Hedgehog signaling. Dev. Dyn. 237, 1359-1372.

Freed, C. R., Greene, P. E., Breeze, R. E., Tsai, W. Y., DuMouchel, W., Kao, R., Dillon, S., Winfield, H., Culver, S., Trojanowski, J. Q., Eidelberg, D. and Fahn, S., 2001. Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N. Engl. J. Med. 344, 710-719.

Freeman, T. B., Cicchetti, F., Hauser, R. A., Deacon, T. W., Li, X. J., Hersch, S. M., Nauert, G. M., Sanberg, P. R., Kordower, J. H., Saporta, S. and Isacson, O., 2000. Transplanted fetal striatum in Huntington's disease: phenotypic development and lack of pathology. Proc. Natl. Acad. Sci. U. S. A. 97, 13877-13882.

Gaillard, A., Decressac, M., Frappe, I., Fernagut, P. O., Prestoz, L., Besnard, S. and Jaber, M., 2009. Anatomical and functional reconstruction of the nigrostriatal pathway by intranigral transplants. Neurobiol. Dis. 35, 477-488.

Gaura, V., Bachoud-Levi, A. C., Ribeiro, M. J., Nguyen, J. P., Frouin, V., Baudic, S., Brugieres, P., Mangin, J. F., Boisse, M. F., Palfi, S., Cesaro, P., Samson, Y., Hantraye, P., Peschanski, M. and Remy, P., 2004. Striatal neural grafting improves cortical metabolism in Huntington's disease patients. Brain 127, 65-72.

Gerber, J. K., Richter, T., Kremmer, E., Adamski, J., Hofler, H., Balling, R. and Peters, H., 2002. Progressive loss of PAX9 expression correlates with increasing malignancy of dysplastic and cancerous epithelium of the human oesophagus. J. Pathol. 197, 293-297.

Gershon, T. R., Oppenheimer, O., Chin, S. S. and Gerald, W. L., 2005. Temporally regulated neural crest transcription factors distinguish neuroectodermal tumors of varying malignancy and differentiation. Neoplasia 7, 575-584.

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. D., Lumsden, A. and Gruss, P., 1993. Signals from the notochord and floor plate regulate the region-specific expression of two Pax genes in the developing spinal cord. Development 117, 1001-1016.

Greene, P. E., Fahn, S., Tsai, W. Y., Breeze, R. E., Eidelberg, D., Winfield, H., Dillon, S., Kao, R., Winfield, L. and Freed, C. R., 1999. Severe spontaneous dyskinesias: a disabling complication of embryonic dopaminergic tissue implants in a subset of transplanted patients with advanced Parkinson's disease. Mov. Disord. 14.

Grindley, J. C., Davidson, D. R. and Hill, R. E., 1995. The role of *Pax-6* in eye and nasal development. Development 121, 1433-1442.

Guo, Z., Packard, A., Krolewski, R. C., Harris, M. T., Manglapus, G. L. and Schwob, J. E., 2010. Expression of pax6 and sox2 in adult olfactory epithelium. J. Comp. Neurol. 518, 4395-4418.

Hack, M. A., Saghatelyan, A., de Chevigny, A., Pfeifer, A., Ashery-Padan, R., Lledo, P. M. and Gotz, M., 2005. Neuronal fate determinants of adult olfactory bulb neurogenesis. Nat. Neurosci. 8, 865-872.

Hack, M. A., Sugimori, M., Lundberg, C., Nakafuku, M. and Gotz, M., 2004. Regionalization and fate specification in neurospheres: the role of Olig2 and Pax6. Mol. Cell. Neurosci. 25, 664-678.

Hadrys, T., DeSalle, R., Sagasser, S., Fischer, N. and Schierwater, B., 2005. The Trichoplax PaxB gene: a putative Proto-PaxA/B/C gene predating the origin of nerve and sensory cells. Mol. Biol. Evol. 22, 1569-1578.

Hamasaki, T., Leingartner, A., Ringstedt, T. and O'Leary, D. D., 2004. EMX2 regulates sizes and positioning of the primary sensory and motor areas in neocortex by direct specification of cortical progenitors. Neuron 43, 359-372.

Haubst, N., Berger, J., Radjendirane, V., Graw, J., Favor, J., Saunders, G. F., Stoykova, A. and Gotz, M., 2004. Molecular dissection of Pax6 function: the specific roles of the paired domain and homeodomain in brain development. Development 131, 6131-6140.

Heine, W., Conant, K., Griffin, J. W. and Hoke, A., 2004. Transplanted neural stem cells promote axonal regeneration through chronically denervated peripheral nerves. Exp. Neurol. 189, 231-240.

Heins, N., Malatesta, P., Cecconi, F., Nakafuku, M., Tucker, K. L., Hack, M. A., Chapouton, P., Barde, Y. A. and Gotz, M., 2002. Glial cells generate neurons: the role of the transcription factor Pax6. Nat. Neurosci. 5, 308-315.

Hill, R. E., Favor, J., Hogan, B. L. M., Ton, C. C. T., 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.

Hirata, H., Tomita, K., Bessho, Y. and Kageyama, R., 2001. Hes1 and Hes3 regulate maintenance of the isthmic organizer and development of the mid/hindbrain. EMBO J. 20, 4454-4466.

Hitchcock, P. F., Macdonald, R. E., VanDeRyt, J. T. and Wilson, S. W., 1996. Antibodies against Pax6 immunostain amacrine and ganglion cells and neuronal progenitors, but not rod precursors, in the normal and regenerating retina of the goldfish. J. Neurobiol. 29, 399-413.

Hodges, H., Sowinski, P., Fleming, P., Kershaw, T. R., Sinden, J. D., Meldrum, B. S. and Gray, J. A., 1996. Contrasting effects of fetal CA1 and CA3 hippocampal grafts on deficits in spatial learning and working memory induced by global cerebral ischaemia in rats. Neuroscience 72, 959-988.

Horie, M., Sango, K., Takeuchi, K., Honma, S., Osumi, N., Kawamura, K. and Kawano, H., 2003. Subpial neuronal migration in the medulla oblongata of Pax-6-deficient rats. Eur. J. Neurosci. 17, 49-57.

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.

Hu, Y. F., Gourab, K., Wells, C., Clewes, O., Schmit, B. D. and Sieber-Blum, M., 2010. Epidermal neural crest stem cell (EPI-NCSC)--mediated recovery of sensory function in a mouse model of spinal cord injury. Stem Cell Rev 6, 186-198.

Imitola, J., Raddassi, K., Park, K. I., Mueller, F. J., Nieto, M., Teng, Y. D., Frenkel, D., Li, J., Sidman, R. L., Walsh, C. A., Snyder, E. Y. and Khoury, S. J., 2004. Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc. Natl. Acad. Sci. U. S. A. 101, 18117-18122.

Isacson, O., 2003. The production and use of cells as therapeutic agents in neurodegenerative diseases. Lancet Neurol 2, 417-424.

Isacson, O., Brundin, P., Kelly, P. A., Gage, F. H. and Bjorklund, A., 1984. Functional neuronal replacement by grafted striatal neurones in the ibotenic acid-lesioned rat striatum. Nature 311, 458-460.

Jain, K. K., 2009. Cell Therapy for CNS Trauma. Mol. Biotechnol. 42, 367-376.

Jimenez, D., Lopez-Mascaraque, L., de Carlos, J. A. and Valverde, F., 2002. Further studies on cortical tangential migration in wild type and Pax-6 mutant mice. J. Neurocytol. 31, 719-728.

Johnson, T. S., O'Neill, A. C., Motarjem, P. M., Nazzal, J., Randolph, M. and Winograd, J. M., 2008. Tumor formation following murine neural precursor cell transplantation in a rat peripheral nerve injury model. J. Reconstr. Microsurg. 24, 545-550.

Jones, L., Lopez-Bendito, G., Gruss, P., Stoykova, A. and Molnar, Z., 2002. *Pax6* is required for the normal development of the forebrain axonal connections. Development 129, 5041-5052.

Jostes, B., Walther, C. and Gruss, P., 1991. The murine paired box gene, Pax7, is expressed specifically during the development of the nervous and muscular system. Mech. Dev. 33, 27-38.

Joyner, A. L., 1996. Engrailed, Wnt and Pax genes regulate midbrain-hindbrain development. Trends Genet. 12, 15-20.

Kallur, T., Gisler, R., Lindvall, O. and Kokaia, Z., 2008. Pax6 promotes neurogenesis in human neural stem cells. Mol. Cell. Neurosci. 38, 616-628.

Kanakubo, S., Nomura, T., Yamamura, K., Miyazaki, J., Tamai, M. and Osumi, N., 2006. Abnormal migration and distribution of neural crest cells in Pax6 heterozygous mutant eye, a model for human eye diseases. Genes Cells 11, 919-933.

Kanteti, R., Nallasura, V., Loganathan, S., Tretiakova, M., Kroll, T., Krishnaswamy, S., Faoro, L., Cagle, P., Husain, A. N., Vokes, E. E., Lang, D. and Salgia, R., 2009. PAX5 is expressed in small-cell lung cancer and positively regulates c-Met transcription. Lab. Invest. 89, 301-314.

Karl, M. O., Hayes, S., Nelson, B. R., Tan, K., Buckingham, B. and Reh, T. A., 2008. Stimulation of neural regeneration in the mouse retina. Proc. Natl. Acad. Sci. U. S. A. 105, 19508-19513.

Kawakami, A., Kimura-Kawakami, M., Nomura, T. and Fujisawa, H., 1997. Distributions of *Pax6* and *Pax7* proteins suggest their involvement in both early and late phases of chick brain development. Mech. Dev. 66, 119-130.

Kawano, O., Fukuda, T., Kubo, K., Horie, M., Uyemura, K., Takeuchi, K., Osumi, N., Eto, K. and Kawamura, K., 1999. *Pax6* is required for thalamocortical pathway formation in fetal rats. J. Comp. Neurol. 408, 147-160.

Kay, P. H. and Ziman, M. R., 1999. Alternate Pax7 paired box transcripts which include a trinucleotide or a hexanucleotide are generated by use of alternate 3' intronic splice sites which are not utilized in the ancestral homologue. Gene 230, 55-60.

Kayama, M., Kurokawa, M. S., Ueda, Y., Ueno, H., Kumagai, Y., Chiba, S., Takada, E., Ueno, S., Tadokoro, M. and Suzuki, N., 2009. Transfection with pax6 Gene of Mouse Embryonic Stem Cells and Subsequent Cell Cloning Induced Retinal Neuron Progenitors, Including Retinal Ganglion Cell-Like Cells, in vitro. Ophthalmic Res. 43, 79-91.

Kioussi, C., Gross, M. K. and Gruss, P., 1995. Pax3: a paired domain gene as a regulator of PNS myelination. Neuron 15, 553-562.

Kippin, T. E., Martens, D. J. and van der Kooy, D., 2005. p21 loss compromises the relative quiescence of forebrain stem cell proliferation leading to exhaustion of their proliferation capacity. Genes Dev. 19, 756-767.

Kohwi, M., Osumi, N., Rubenstein, J. L. R. and Alvarez-Buylla, A., 2005. Pax6 is required for making specific subpopulations of granule and periglomerular neurons in the olfactory bulb. J. Neurosci. 25, 6997-7003.

Kordower, J. H., Chu, Y., Hauser, R. A., Freeman, T. B. and Olanow, C. W., 2008a. Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat. Med. 14, 504-506.

Kordower, J. H., Chu, Y., Hauser, R. A., Olanow, C. W. and Freeman, T. B., 2008b. Transplanted dopaminergic neurons develop PD pathologic changes: a second case report. Mov. Disord. 23, 2303-2306.

Kordower, J. H., Freeman, T. B., Snow, B. J., Vingerhoets, F. J., Mufson, E. J., Sanberg, P. R., Hauser, R. A., Smith, D. A., Nauert, G. M., Perl, D. P. and et al., 1995. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. N. Engl. J. Med. 332, 1118-1124.

Kozmik, Z., Sure, U., Ruedi, D., Busslinger, M. and Aguzzi, A., 1995. Deregulated expression of PAX5 in medulloblastoma. Proc. Natl. Acad. Sci. U. S. A. 92, 5709-5713.

Kukekov, V. G., Laywell, E. D., Suslov, O., Davies, K., Scheffler, B., Thomas, L. B., O'Brien, T. F., Kusakabe, M. and Steindler, D. A., 1999. Multipotent stem/progenitor cells with similar properties arise from two neurogenic regions of adult human brain. Exp. Neurol. 156, 333-344.

Lamey, T. M., Koenders, A. and Ziman, M., 2004. Pax genes in myogenesis: alternate transcripts add complexity. Histol. Histopathol. 19, 1289-1300.

Lang, D., Lu, M. M., Huang, L., Engleka, K. A., Zhang, M., Chu, E. Y., Lipner, S., Skoultchi, 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 Belle, J. E., Caldwell, M. A. and Svendsen, C. N., 2004. Improving the survival of human CNS precursor-derived neurons after transplantation. J. Neurosci. Res. 76, 174-183.

Leconte, L., Lecoin, L., Martin, P. and Saule, S., 2004. Pax6 interacts with cVax and Tbx5 to establish the dorsoventral boundary of the developing eye. J. Biol. Chem. 279, 47272-47277.

Li, J. Y., Englund, E., Holton, J. L., Soulet, D., Hagell, P., Lees, A. J., Lashley, T., Quinn, N. P., Rehncrona, S., Bjorklund, A., Widner, H., Revesz, T., Lindvall, O. and Brundin, P., 2008. Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat. Med. 14, 501-503.

Li, N., Hornbruch, A., Klafke, R., Katzenberger, B. and Wizenmann, A., 2005. Specification of dorsoventral polarity in the embryonic chick mesencephalon and its presumptive role in midbrain morphogenesis. Dev. Dyn. 233, 907-920.

Li, Y., Chen, J., Wang, L., Lu, M. and Chopp, M., 2001. Treatment of stroke in rat with intracarotid administration of marrow stromal cells. Neurology 56, 1666-1672.

Liem, K. F. J., Tremml, G., Roelink, H. and Jessell, T. M., 1995. Dorsal differentiation of neural plate cells induced by BMP-mediated signals from epidermal ectoderm. Cell 82, 969-979.

Lindvall, O. and Hagell, P., 2000. Clinical observations after neural transplantation in Parkinson's disease. Prog. Brain Res. 127, 299-320.

Lu, D., Mahmood, A., Wang, L., Li, Y., Lu, M. and Chopp, M., 2001. Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome. Neuroreport 12, 559-563.

Lumsden, A. and Krumlauf, R., 1996. Patterning the vertebrate neuraxis. Science 274, 1109-1115.

Lun, K. and Brand, M., 1998. A series of *no isthmus (noi)* alleles of the zebrafish *pax2.1* gene reveals multiple signalling events in development of the midbrain-hindbrain boundary. Development 125, 3049-3062.

Maekawa, M., Takashima, N., Arai, Y., Nomura, T., Inokuchi, K., Yuasa, S. and Osumi, N., 2005. Pax6 is required for production and maintenance of progenitor cells in postnatal hippocampal neurogenesis. Genes Cells 10, 1001-1014.

Mahmood, A., Lu, D., Wang, L., Li, Y., Lu, M. and Chopp, M., 2001. Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells. Neurosurgery 49, 1196-1203; discussion 1203-1194.

Majdan, M., Lachance, C., Gloster, A., Aloyz, R., Zeindler, C., Bamji, S., Bhakar, A., Belliveau, D., Fawcett, J., Miller, F. D. and Barker, P. A., 1997. Transgenic mice expressing the intracellular domain of the p75 neurotrophin receptor undergo neuronal apoptosis. J. Neurosci. 17, 6988-6998.

Mansouri, A., Stoykova, A., Torres, M. and Gruss, P., 1996. Dysgenesis of cephalic neural crest derivatives in Pax7-/- mutant mice. Development 122, 831-838.

Manuel, M., Georgala, P. A., Carr, C. B., Chanas, S., Kleinjan, D. A., Martynoga, B., Mason, J. O., Molinek, M., Pinson, J., Pratt, T., Quinn, J. C., Simpson, T. I., Tyas, D. A., van Heyningen, V., West, J. D. and Price, D. J., 2007. Controlled overexpression of Pax6 in vivo negatively autoregulates the Pax6 locus, causing cell-autonomous defects of late cortical progenitor proliferation with little effect on cortical arealization. Development 134, 545-555.

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. and Andrews, G. L., 2001. Pax6 regulates the identity of embryonic diencephalic neurons. Mol. Cell. Neurosci. 17, 190-207.

Matsunaga, E., Araki, I. and Nakamura, H., 2000. *Pax6* defines the di-mesencephalic boundary by repressing *En1* and *Pax2*. Development 127, 2357-2365.

Matsunaga, E., Araki, I. and Nakamura, H., 2001. Role of *Pax3/7* in the tectum regionalization. Development 128, 4069-4077.

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., George, D., Mania-Farnell, B., Bremer, C. L., McLone, D. G. and Bremer, E. G., 2000. Overexpression of murine Pax3 increases NCAM polysialylation in a human medulloblastoma cell line. J. Biol. Chem. 275, 23259-23266.

Medic, S. and Ziman, M., 2009. PAX3 across the spectrum: from melanoblast to melanoma. Crit. Rev. Biochem. Mol. Biol., 1-13.

Meech, R., Kallunki, P., Edelman, G. M. and Jones, F. S., 1999. A binding site for homeodomain and Pax proteins is necessary for L1 cell adhesion molecule gene expression by Pax-6 and bone morphogenetic proteins. Proc. Natl. Acad. Sci. U. S. A. 96, 2420-2425.

Mine, Y., Hayashi, T., Yamada, M., Okano, H. and Kawase, T., 2009. Environmental cue-dependent dopaminergic neuronal differentiation and functional effect of grafted neuroepithelial stem cells in parkinsonian brain. Neurosurgery 65, 741-753; discussion 753.

Mo, Z. and Zecevic, N., 2008. Is Pax6 critical for neurogenesis in the human fetal brain? Cereb. Cortex 18, 1455-1465.

Monsoro-Burq, A.-H., Duprez, D., Watanabe, Y., Bontoux, M., Vincent, C., Brickell, P. and Le Douarin, N., 1996. The role of bone morphogenetic proteins in vertebral development. Development 122, 3607-3616.

Muratovska, A., Zhou, C., He, S., Goodyer, P. and Eccles, M. R., 2003. Paired-Box genes are frequently expressed in cancer and often required for cancer cell survival. Oncogene 22, 7989-7997.

Murdoch, B., DelConte, C. and Garcia-Castro, M. I., 2010. Embryonic Pax7-expressing progenitors contribute multiple cell types to the postnatal olfactory epithelium. J. Neurosci. 30, 9523-9532.

Muzio, L. and Mallamaci, A., 2003. Emx1, emx2 and pax6 in specification, regionalization and arealization of the cerebral cortex. Cereb. Cortex 13, 641-647.

Nacher, J., Varea, E., Blasco-Ibanez, J. M., Castillo-Gomez, E., Crespo, C., Martinez-Guijarro, F. J. and McEwen, B. S., 2005. Expression of the transcription factor Pax 6 in the adult rat dentate gyrus. J. Neurosci. Res. 81, 753-761.

Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., Tamura, A., Kirino, T. and Nakafuku, M., 2002. Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 110, 429-441.

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.

Nebral, K., Konig, M., Harder, L., Siebert, R., Haas, O. A. and Strehl, S., 2007. Identification of PML as novel PAX5 fusion partner in childhood acute lymphoblastic leukaemia. Br. J. Haematol. 139, 269-274.

Neubuser, A., Koseki, H. and Balling, R., 1995. Characterization and developmental expression of Pax9, a paired-box-containing gene related to Pax1. Dev. Biol. 170, 701-716.

Newman, M. B., Willing, A. E., Manresa, J. J., Davis-Sanberg, C. and Sanberg, P. R., 2005. Stroke-induced migration of human umbilical cord blood cells: time course and cytokines. Stem Cells Dev 14, 576-586.

Nikoletopoulou, V., Plachta, N., Allen, N. D., Pinto, L., Gotz, M. and Barde, Y. A., 2007. Neurotrophin receptor-mediated death of misspecified neurons generated from embryonic stem cells lacking Pax6. Cell Stem Cell 1, 529-540.

Ninkovic, J., Pinto, L., Petricca, S., Lepier, A., Sun, J., Rieger, M. A., Schroeder, T., Cvekl, A., Favor, J. and Gotz, M., 2010. The transcription factor Pax6 regulates survival of dopaminergic olfactory bulb neurons via crystallin alphaA. Neuron 68, 682-694.

Nomura, T., Kawakami, A. and Fujisawa, H., 1998. Correlation between tectum formation and expression of two *PAX* family genes, *PAX7* and *PAX6*, in avian brains. Development, Growth and Differentiation 40, 485-495.

Nornes, H. O., Dressler, G. R., Knapik, E. W., Deutsch, U. and Gruss, P., 1990. Spatially and temporally restricted expression of Pax2 during murine neurogenesis. Development 109, 797-809.

Nutt, S. L., Morrison, A. M., Dorfler, P., Rolink, A. and Busslinger, M., 1998. Identification of BSAP (Pax-5) target genes in early B-cell development by loss- and gain-of-function experiments. EMBO J. 17, 2319-2333.

Osumi-Yamashita, N., Kuratani, S., Ninomiya, Y., Aoki, K., Iseki, S., Chareonvit, S., Doi, H., Fujiwara, M., Watanabe, T. and Eto, K., 1997. Cranial anomaly of homozygous rSey rat is associated with a defect in the migration pathway of midbrain crest cells. Dev. Growth Differ. 39, 53-67.

Otto, A., Schmidt, C. and Patel, K., 2006. Pax3 and Pax7 expression and regulation in the avian embryo. Anat. Embryol. (Berl). 211, 293-310.

Pani, L., Horal, M. and Loeken, M. R., 2002. Rescue of neural tube defects in Pax-3-deficient embryos by p53 loss of function: implications for Pax-3-dependent development and tumorigenesis. Genes Dev. 16, 676-680.

Park, D., Jia, H., Rajakumar, V. and Chamberlin, H. M., 2006. Pax2/5/8 proteins promote cell survival in C. elegans. Development 133, 4193-4202.

Park, D. H., Eve, D. J., Musso, J. r., Klasko, S. K., Cruz, E., Borlongan, C. V. and Sanberg, P. R., 2009. Inflammation and stem cell migration to the injured brain in higher organisms. Stem Cells Dev 18, 693-702.

Pera, M. F. and Tam, P. P., 2010. Extrinsic regulation of pluripotent stem cells. Nature 465, 713-720.

Peters, H., Neubuser, A., Kratochwil, K. and Balling, R., 1998. Pax9-deficient mice lack pharyngeal pouch derivatives and teeth and exhibit craniofacial and limb abnormalities. Genes Dev. 12, 2735-2747.

Pfeffer, P. L., Gerster, T., Lun, K., Brand, M. and Busslinger, M., 1998. Characterization of three novel members of the zebrafish *Pax2/5/8* family: dependency of *Pax5* and *Pax8* expression on the *Pax2.1 (noi)* function. Development 125, 3063-3074.

Phelan, S. A., Ito, M. and Leoken, M. B., 1997. Neural Tube Defects in embryos of diabetic mice: Role of the Pax3 gene and apoptosis. Diabetes 46, 1189-1197.

Philips, G. T., Stair, C. N., Lee, H. Y., Wroblewski, E., Berberoglu, M. A., Brown, N. L. and Mastick, G. S., 2005. Precocious retinal neurons: *Pax6* controls timing of differentiation and determination of cell type. Dev. Biol. 279, 308-321.

Pillai, A., Mansouri, A., Behringer, R., Westphal, H. and Goulding, M., 2007. Lhx1 and Lhx5 maintain the inhibitory-neurotransmitter status of interneurons in the dorsal spinal cord. Development 134, 357-366.

Pinon, M. C., Tuoc, T. C., Ashery-Padan, R., Molnar, Z. and Stoykova, A., 2008. Altered molecular regionalization and normal thalamocortical connections in cortex-specific Pax6 knock-out mice. J. Neurosci. 28, 8724-8734.

Pinson, J., Mason, J. O., Simpson, T. I. and Price, D. J., 2005. Regulation of the Pax6 : Pax6(5a) mRNA ratio in the developing mammalian brain. BMC Dev Biol 5, 13.

Pinson, J., Simpson, T. I., Mason, J. O. and Price, D. J., 2006. Positive autoregulation of the transcription factor Pax6 in response to increased levels of either of its major isoforms, Pax6 or Pax6(5a), in cultured cells. BMC Dev Biol 6, 25.

Plachta, N., Annaheim, C., Bissiere, S., Lin, S., Ruegg, M., Hoving, S., Muller, D., Poirier, F., Bibel, M. and Barde, Y. A., 2007. Identification of a lectin causing the degeneration of neuronal processes using engineered embryonic stem cells. Nat. Neurosci. 10, 712-719.

Plaza, S., Saule, S. and Dozier, C., 1999. High conservation of cis-regulatory elements between quail and human for the Pax-6 gene. Dev. Genes Evol. 209, 165-173.

Pluchino, S., Cusimano, M., Bacigaluppi, M. and Martino, G., 2010. Remodelling the injured CNS through the establishment of atypical ectopic perivascular neural stem cell niches. Arch. Ital. Biol. 148, 173-183.

Ponti, G., Peretto, P. and Bonfanti, L., 2008. Genesis of neuronal and glial progenitors in the cerebellar cortex of peripuberal and adult rabbits. PLoS One 3, e2366.

Pratt, T., Quinn, J. C., Simpson, T. I., West, J. D., Mason, J. O. and Price, D. J., 2002. Disruption of early events in thalamocortical tract formation in mice lacking the transcription factors Pax6 or Foxg1. J. Neurosci. 22, 8523-8531.

Pritzel, M., Isacson, O., Brundin, P., Wiklund, L. and Bjorklund, A., 1986. Afferent and efferent connections of striatal grafts implanted into the ibotenic acid lesioned neostriatum in adult rats. Exp. Brain Res. 65, 112-126.

Radtke, N. D., Aramant, R. B., Petry, H. M., Green, P. T., Pidwell, D. J. and Seiler, M. J., 2008. Vision improvement in retinal degeneration patients by implantation of retina together with retinal pigment epithelium. Am. J. Ophthalmol. 146, 172-182.

Radtke, N. D., Aramant, R. B., Seiler, M. J., Petry, H. M. and Pidwell, D., 2004. Vision change after sheet transplant of fetal retina with retinal pigment epithelium to a patient with retinitis pigmentosa. Arch. Ophthalmol. 122, 1159-1165.

Real, C., Glavieux-Pardanaud, C., Le Douarin, N. M. and Dupin, E., 2006. Clonally cultured differentiated pigment cells can dedifferentiate and generate multipotent progenitors with self-renewing potential. Dev. Biol. 300, 656-669.

Redies, C. and Puelles, L., 2001. Modularity in vertebrate brain development and evolution. Bioessays 23, 1100-1111.

Reeves, F. C., Burdge, G. C., Fredericks, W. J., Rauscher, F. J. I. and Lillycrop, K. A., 1999. Induction of antisense Pax-3 expression leads to the rapid morphological differentiation of neuronal cells and an altered response to the mitogenic growth factor bFGF. J. Cell Sci. 112, 253-261.

Reubinoff, B. E., Itsykson, P., Turetsky, T., Pera, M. F., Reinhartz, E., Itzik, A. and Ben-Hur, T., 2001. Neural progenitors from human embryonic stem cells. Nat. Biotechnol. 19, 1134-1140.

Robson, E. J., He, S. J. and Eccles, M. R., 2006. A PANorama of PAX genes in cancer and development. Nat Rev Cancer 6, 52-62.

Saghatelyan, A., de Chevigny, A., Schachner, M. and Lledo, P. M., 2004. Tenascin-R mediates activity-dependent recruitment of neuroblasts in the adult mouse forebrain. Nat. Neurosci. 7, 347-356.

Sansom, S. N., Griffiths, D. S., Faedo, A., Kleinjan, 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.

Scardigli, R., Baumer, N., Gruss, P., Guillemot, F. and Le Roux, I., 2003. Direct and concentration-dependent regulation of the proneural gene Neurogenin2 by Pax6. Development 130, 3269-3281.

Schwarz, M., Alvarez-Bolado, G., Dressler, G., Urbanek, P., Busslinger, M. and Gruss, P., 1999. Pax 2/5 and Pax6 subdivide the early neural tube into three domains. Mech. Dev. 82, 29-39.

Schwarz, M., Cecconi, F., Bernier, G., Andrejewski, N., Kammandel, B., Wagner, M. and Gruss, P., 2000. Spatial specification of mammalian eye territories by reciprocal transcriptional repression of *Pax2* and *Pax6*. Development 127, 4325-4334.

Shimizu, N., Watanabe, H., Kubota, J., Wu, J., Saito, R., Yokoi, T., Era, T., Iwatsubo, T., Watanabe, T., Nishina, S., Azuma, N., Katada, T. and Nishina, H., 2009. Pax6-5a promotes neuronal differentiation of murine embryonic stem cells. Biol. Pharm. Bull. 32, 999-1003.

Shin, D. H., Lee, K.-S., Lee, E., Chang, Y. A., J-W, K., Choi, Y. S., Kwon, B.-S., Lee, H. W. and Cho, S. S., 2003. Pax-7 immunoreactivity in the postnatal chicken central nervous system. Anatomical and Histological Embryology 32, 378-383.

Sieber-Blum, M., 2010. Epidermal neural crest stem cells and their use in mouse models of spinal cord injury. Brain Res. Bull. 83, 189-193.

Sorensen, J. C., Grabowski, M., Zimmer, J. and Johansson, B. B., 1996. Fetal neocortical tissue blocks implanted in brain infarcts of adult rats interconnect with the host brain. Exp. Neurol. 138, 227-235.

Soukkarieh, C., Agius, E., Soula, C. and Cochard, P., 2007. Pax2 regulates neuronal-glial cell fate choice in the embryonic optic nerve. Dev. Biol. 303, 800-813.

Spitere, K., Toulouse, A., O'Sullivan, D. B. and Sullivan, A. M., 2008. TAT-PAX6 protein transduction in neural progenitor cells: a novel approach for generation of dopaminergic neurones in vitro. Brain Res. 1208, 25-34.

Srivastava, A. S., Malhotra, R., Sharp, J. and Berggren, T., 2008. Potentials of ES cell therapy in neurodegenerative diseases. Curr. Pharm. Des. 14, 3873-3879.

Steinbach, J. P., Kozmik, Z., Pfeffer, P. and Aguzzi, A., 2001. Overexpression of Pax5 is not sufficient for neoplastic transformation of mouse neuroectoderm. Int. J. Cancer 93, 459-467.

Stoykova, A. and Gruss, P., 1994. Roles of Pax-genes in developing and adult brain as suggested by expression patterns. J. Neurosci. 14, 1395-1412.

Stuart, E. T., Haffner, R., Oren, M. and Gruss, P., 1995a. Loss of p53 function through PAX-mediated transcriptional repression. EMBO J. 14, 5638-5645.

Stuart, E. T., Kioussi, C., Aguzzi, A. and Gruss, P., 1995b. PAX5 expression correlates with increasing malignancy in human astrocytomas. Clin. Cancer Res. 1, 207-214.

Sugimori, M., Nagao, M., Bertrand, N., Parras, C. M., Guillemot, F. and Nakafuku, M., 2007. Combinatorial actions of patterning and HLH transcription factors in the spatiotemporal control of neurogenesis and gliogenesis in the developing spinal cord. Development 134, 1617-1629.

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.

Talamillo, A., Quinn, J. C., Collinson, J. M., Caric, D., Price, D. J., West, J. D. and Hill, R. E., 2003. Pax6 regulates regional development and neuronal migration in the cerebral cortex. Dev. Biol. 255, 151-163.

Thomas, M., Tyers, P., Lazic, S. E., Barker, R. A., Beazley, L. and Ziman, M., 2009. Graft outcomes influenced by co-expression of Pax7 in graft and host tissue. J. Anat. 214, 396-405.

Thomas, M. G., Barker, R. A., Beazley, L. D. and Ziman, M. R., 2007. Pax7 expression in the adult rat superior colliculus following optic nerve injury. Neuroreport 18, 105-109.

Thompson, J. A., Lovicu, F. J. and Ziman, M., 2007. Pax7 and superior collicular polarity: insights from Pax6 (Sey) mutant mice. Exp. Brain Res. 178, 316-325.

Thompson, J. A., Zembrzycki, A., Mansouri, A. and Ziman, M., 2008. *Pax7* is requisite for maintenance of a subpopulation of superior collicular neurons and shows a diverging expression pattern to *Pax3* during superior collicular development. Dev. Biol. 8, 62.

Thompson, L. H., Grealish, S., Kirik, D. and Bjorklund, A., 2009. Reconstruction of the nigrostriatal dopamine pathway in the adult mouse brain. Eur. J. Neurosci. 30, 625-638.

Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S. and Jones, J. M., 1998. Embryonic stem cell lines derived from human blastocysts. Science 282, 1145-1147.

Tonchev, A. B. and Yamashima, T., 2006. Differential neurogenic potential of progenitor cells in dentate gyrus and CA1 sector of the postischemic adult monkey hippocampus. Exp. Neurol. 198, 101-113.

Tonchev, A. B., Yamashima, T., Sawamoto, K. and Okano, H., 2006. Transcription factor protein expression patterns by neural or neuronal progenitor cells of adult monkey subventricular zone. Neuroscience 139, 1355-1367.

Tong, G. X., Weeden, E. M., Hamele-Bena, D., Huan, Y., Unger, P., Memeo, L. and O'Toole, K., 2008. Expression of PAX8 in nephrogenic adenoma and clear cell adenocarcinoma of the lower urinary tract: evidence of related histogenesis? Am. J. Surg. Pathol. 32, 1380-1387.

Torres, M., Gomez-Pardo, E. and Gruss, P., 1996. Pax2 contributes to inner ear patterning and optic nerve trajectory. Development 122, 3381-3391.

Treisman, J., Harris, E. and Desplan, C., 1991. The paired box encodes a second DNA-binding domain in the paired homeo domain protein. Genes Dev. 5, 594-604.

Tuoc, T. C., Radyushkin, K., Tonchev, A. B., Pinon, M. C., Ashery-Padan, R., Molnar, Z., Davidoff, M. S. and Stoykova, A., 2009. Selective cortical layering abnormalities and behavioral deficits in cortex-specific Pax6 knock-out mice. J. Neurosci. 29, 8335-8349.

Ueno, H., Kurokawa, M. S., Kayama, M., Homma, R., Kumagai, Y., Masuda, C., Takada, E., Tsubota, K., Ueno, S. and Suzuki, N., 2007. Experimental transplantation of corneal epithelium-like cells induced by Pax6 gene transfection of mouse embryonic stem cells. Cornea 26, 1220-1227.

Underhill, D. A. and Gros, P., 1997. The paired-domain regulates DNA binding by the homeodomain within the intact Pax-3 protein. J. Biol. Chem. 272, 14175-14182.

Underwood, T. J., Amin, J., Lillycrop, K. A. and Blaydes, J. P., 2007. Dissection of the functional interaction between p53 and the embryonic proto-oncoprotein PAX3. FEBS Lett. 581, 5831-5835.

Vogan, K. J. and Gros, P., 1997. The C-terminal subdomain makes an important contribution to the DNA binding activity of the Pax-3 paired domain. J. Biol. Chem. 272, 28289-28295.

Vogan, K. J., Underhill, D. A. and Gros, P., 1996. An alternative splicing event in the Pax-3 paired domain identifies the linker region as a key determinant of paired domain DNA-binding activity. Mol. Cell. Biol. 16, 6677-6686.

Vorobyov, E. and Horst, J., 2006. Getting the proto-Pax by the tail. J. Mol. Evol. 63, 153-164.

Wallin, J., Eibel, H., Neubuser, A., Wilting, 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.

Walther, C., Guenet, J. L., Simon, D., Deutsch, U., Jostes, B., Goulding, M. D., Plachov, D., Balling, R. and Gruss, P., 1991. Pax: a murine multigene family of paired box-containing genes. Genomics 11, 424-434.

Wang, Q., Fang, W. H., Krupinski, J., Kumar, S., Slevin, M. and Kumar, P., 2008. Pax genes in embryogenesis and oncogenesis. J Cell Mol Med 12, 2281-2294.

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, 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.

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.

Wernig, M., Benninger, F., Schmandt, T., Rade, M., Tucker, K. L., Bussow, H., Beck, H. and Brustle, O., 2004. Functional integration of embryonic stem cell-derived neurons in vivo. J. Neurosci. 24, 5258-5268.

White, R. B. and Ziman, M. R., 2008. Genome-wide discovery of Pax7 target genes during development. Physiol Genomics 33, 41-49.

Wictorin, K., Brundin, P., Gustavii, B., Lindvall, O. and Bjorklund, A., 1990. Reformation of long axon pathways in adult rat central nervous system by human forebrain neuroblasts. Nature 347, 556-558.

Winkler, C., Kirik, D., Bjorklund, A. and Dunnett, S. B., 2000. Transplantation in the rat model of Parkinson's disease: ectopic versus homotopic graft placement. Prog. Brain Res. 127, 233-265.

Yamamoto, S.-i., Nagao, M., Sugimori, M., Kosako, H., Nakatomi, H., Yamamoto, N., Takebayashi, H., Nabeshima, Y.-i., Kitamura, T., Weinmaster, G., Nakamura, K. and Nakafuku, M., 2001. Transcription factor expression and Notch-dependent regulation of neural progenitors in the adult rat spinal cord. J. Neurosci. 21, 9814-9823.

Yang, G., Li, Y., Nishimura, E. K., Xin, H., Zhou, A., Guo, Y., Dong, L., Denning, M. F., Nickoloff, B. J. and Cui, R., 2008. Inhibition of PAX3 by TGF-beta modulates melanocyte viability. Mol. Cell 32, 554-563.

Yang, K., Jiang, Z., Wang, D., Lian, X. and Yang, T., 2009. Corneal epithelial-like transdifferentiation of hair follicle stem cells is mediated by pax6 and beta-catenin/Lef-1. Cell Biol. Int. 33, 861-866.

Yang, P. B., Seiler, M. J., Aramant, R. B., Yan, F., Mahoney, M. J., Kitzes, L. M. and Keirstead, H. S., 2010. Trophic factors GDNF and BDNF improve function of retinal sheet transplants. Exp. Eye Res. 91, 727-738.

Zaghloul, N. A. and Moody, S. A., 2007. Alterations of rx1 and pax6 expression levels at neural plate stages differentially affect the production of retinal cell types and maintenance of retinal stem cell qualities. Dev. Biol. 306, 222-240.

Zhang, J., Lu, J. P., Suter, D. M., Krause, K. H., Fini, M. E., Chen, B. and Lu, Q., 2010. Isoform- and dose-sensitive feedback interactions between paired box 6 gene and delta-catenin in cell differentiation and death. Exp. Cell Res. 316, 1070-1081.

Zhou, H. M., Wang, J., Rogers, R. and Conway, S. J., 2008. Lineage-specific responses to reduced embryonic Pax3 expression levels. Dev. Biol. 315, 369-382.

Ziman, M. R., Fletcher, S. and Kay, P. H., 1997. Alternate Pax7 transcripts are expressed specifically in skeletal muscle, brain and other organs of adult mice. Int. J. Biochem. Cell Biol. 29, 1029-1036.

Ziman, M. R. and Kay, P. H., 1998. Differential expression of four alternate Pax7 paired box transcripts is influenced by organ- and strain-specific factors in adult mice. Gene 217, 77-81.

Ziman, M. R., Rodger, J., Chen, P., Papdimitriou, J. M., Dunlop, S. A. and Beazley, L. D., 2001a. *Pax* genes in development and maturation of the vertebrate visual system: Implications for optic nerve regeneration. Histol. Histopathol. 16, 239-249.

Ziman, M. R., Thomas, M., Jacobsen, P. and Beazley, L., 2001b. A key role for Pax7 transcripts in determination of muscle and nerve cells. Exp. Cell Res. 268, 220-229.