2011

Pax genes during neural development and their potential role in neuroregeneration

Jennifer A. Thompson  
*Edith Cowan University*

Melanie Ziman  
*Edith Cowan University*

PAX GENES DURING NEURAL DEVELOPMENT AND THEIR POTENTIAL ROLE IN NEUROREGENERATION

Jennifer A. Thompson\textsuperscript{a} & Mel Ziman\textsuperscript{a, b}

\textsuperscript{a}School of Medical Sciences, Edith Cowan University, 270 Joondalup Drive, Joondalup, Perth, Western Australia 6027, Australia.

\textsuperscript{b}School of Pathology and Laboratory Medicine, University of Western Australia, Crawley, Perth, Western Australia, Australia.

Email: jennifer.thompson@ecu.edu.au

Corresponding author: Email: jennifer.thompson@ecu.edu.au

Telephone (61 8) 6304 5635

Fax (61 8) 6304 5717
**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.
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.** 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

*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 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 (e.g., 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<sup>−/−</sup> 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.

<table>
<thead>
<tr>
<th>Region</th>
<th><em>Pax</em> Gene</th>
<th>Expression and Potential Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forebrain</td>
<td><em>Pax2</em></td>
<td>Forebrain/midbrain boundary (with En-1) (opposes <em>Pax</em>)</td>
<td>(Matsunaga <em>et al</em>., 2000; Schwarz <em>et al</em>., 1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specification of precursor cells of the forebrain, eye field, otic vesicle</td>
<td>(Fotaki <em>et al</em>., 2008; Nornes <em>et al</em>., 1990; Torres <em>et al</em>., 1996)</td>
</tr>
<tr>
<td></td>
<td><em>Pax6</em></td>
<td>Forebrain/midbrain boundary formation (opposes <em>Pax2</em>)</td>
<td>(Matsunaga <em>et al</em>., 2000; Schwarz <em>et al</em>., 1999)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specification of cortical progenitors, and cortical arealisation (opposes <em>Emx2</em>)</td>
<td>(Bishop <em>et al</em>., 2000; Gotz <em>et al</em>., 1998)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Specification of neural plate cells of the eye field/dorsoventral patterning of the retina</td>
<td>(Leconte <em>et al</em>., 2004; Zaghloul and Moody, 2007)</td>
</tr>
<tr>
<td></td>
<td><em>Pax7</em></td>
<td>Specification of hypothalamic progenitor cells</td>
<td>(Ohyama <em>et al</em>., 2008)</td>
</tr>
<tr>
<td>Midbrain</td>
<td><em>Pax 2/5/8</em></td>
<td>Midbrain/hindbrain boundary formation and polarisation regulated by the isthmic organiser</td>
<td>(Brand <em>et al</em>., 1996; Lun and Brand, 1998; Pfeffer <em>et al</em>., 1998; Picker <em>et al</em>., 1999; Rowitch and McMahon, 1995)</td>
</tr>
<tr>
<td></td>
<td><em>Pax6</em></td>
<td>Expressed in ventral embryonic midbrain and adult dorsolateral substantia nigra reticularis</td>
<td>(Stoykova and Gruss, 1994)</td>
</tr>
<tr>
<td></td>
<td><em>Pax3</em></td>
<td>Specification of the dorsal mesencephalon and is restricted to undifferentiated mesencephalic cells after boundary formation</td>
<td>(Matsunaga <em>et al</em>., 2001; Stoykova and Gruss, 1994; Thompson <em>et al</em>., 2008)</td>
</tr>
<tr>
<td></td>
<td><em>Pax7</em></td>
<td>Specification of the dorsal mesencephalon, and is expressed in undifferentiated and differentiated (neuronal) mesencephalic cells after boundary formation</td>
<td>(Kawakami <em>et al</em>., 1997; Matsunaga <em>et al</em>., 2001; Nomura <em>et al</em>., 1998; Stoykova and Gruss, 1994; Thompson <em>et al</em>., 2007; Thompson <em>et al</em>., 2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polarisation of the dorsal mesencephalon</td>
<td>(Thomas <em>et al</em>., 2007; Thompson <em>et al</em>., 2007)</td>
</tr>
</tbody>
</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.

<table>
<thead>
<tr>
<th>Pax Gene</th>
<th>Patterning and structural abnormalities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pax2</strong></td>
<td><strong>Loss of the isthmus</strong>; failure to close the midbrain neural tube, abnormalities of the midbrain/hindbrain region</td>
<td>(Brand et al., 1996; Torres et al., 1996)</td>
</tr>
<tr>
<td></td>
<td><strong>Visual defects</strong>; 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</td>
<td>(Torres et al., 1996)</td>
</tr>
<tr>
<td></td>
<td><strong>Auditory defects</strong>; abnormal epithelial morphogenesis producing defects of the cochlear and spiral ganglion of the inner ear</td>
<td>(Christophorou et al., 2010; Torres et al., 1996)</td>
</tr>
<tr>
<td><strong>Pax5</strong></td>
<td><strong>Midbrain defects</strong>; reduction of the inferior colliculus</td>
<td>(Urbanek et al., 1994)</td>
</tr>
<tr>
<td></td>
<td><strong>Hindbrain defects</strong>; altered foliation of the anterior cerebellum</td>
<td></td>
</tr>
<tr>
<td><strong>Pax6</strong></td>
<td><strong>Cortical defects</strong>; 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</td>
<td>(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)</td>
</tr>
<tr>
<td></td>
<td><strong>Visual defects</strong>; Microphthalmia (Pax6 over-/under-expression) or anophthalmia (absence of Pax6); dorsalisation of the retina (Pax6 overexpression), ventralisation of the retina (absence of Pax6)</td>
<td>(Baumer et al., 2002; Hill et al., 1991; Hogan et al., 1986; Leconte et al., 2004; Manuel et al., 2007)</td>
</tr>
<tr>
<td></td>
<td><strong>Craniofacial defects</strong>; Required for differentiation of nasal placodes; Pax6 null mice exhibit an imperforate snout</td>
<td>(Hill et al., 1991; Hogan et al., 1986)</td>
</tr>
<tr>
<td><strong>Pax2/Pax6#</strong></td>
<td><strong>Visual defects</strong>; Transdifferentiation of retinal pigmented epithelium into neuroretina</td>
<td>(Baumer et al., 2003)</td>
</tr>
<tr>
<td><strong>Pax3</strong></td>
<td><strong>Neural tube defects</strong>; Open neural folds, neural tube irregularities and exencephaly (Embryonic lethal)</td>
<td>(Auerbach, 1954)</td>
</tr>
<tr>
<td></td>
<td><strong>Neural crest defects</strong>; Cardiac defect - conotruncal defect due to reduction in migratory cardiac neural crest cells</td>
<td>(Conway et al., 2000)</td>
</tr>
<tr>
<td><strong>Pax7</strong></td>
<td><strong>Midbrain defects</strong>; Failure to maintain a subpopulation of dorsal mesencephalic neurons</td>
<td>(Thompson et al., 2008)</td>
</tr>
<tr>
<td></td>
<td><strong>Craniofacial defects</strong>; Absence of nasal capsule and reduction in maxilla and tubules of nasal serous glands due to aberrant neural crest cell specification</td>
<td>(Mansouri et al., 1996)</td>
</tr>
<tr>
<td><strong>Pax3/Pax7#</strong></td>
<td><strong>Neural tube and spinal cord defects</strong>; extensive exencephaly/spina bifida/ventralisation of dorsal spinal cord interneurons (Embryonic lethal)</td>
<td>(Mansouri and Gruss, 1998)</td>
</tr>
</tbody>
</table>
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<sup>−/−</sup> 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 \( Hmg2 \)), 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

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

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 p21cip1, p27kip1, p57Kip2) 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.,
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 astrogensis (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-\(\alpha\)A (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

<table>
<thead>
<tr>
<th>Pax Gene</th>
<th>Adult animal cells showing Pax expression</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pax2</td>
<td>GABAergic cerebellar interneurons (rabbit)</td>
<td>(Ponti et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Nuclei of the midbrain, pons/medulla and cerebellum (mouse)</td>
<td>(Stoykova and Gruss, 1994)</td>
</tr>
<tr>
<td>Pax6</td>
<td>Retinal cells, telencephalon, diencephalon, ventral mesencephalon, cerebellum and pons/medulla (various mammalian species)</td>
<td>(Nacher et al., 2005; Stanescu et al., 2007; Stoykova and Gruss, 1994)</td>
</tr>
<tr>
<td></td>
<td>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</td>
<td>(Guo et al., 2010; Hack et al., 2005; Kohwi et al., 2005; Maekawa et al., 2005; Nacher et al., 2005; Yamamoto et al., 2001)</td>
</tr>
<tr>
<td>Pax3</td>
<td>Bergmann glia and cells surrounding Purkinje cells of the cerebellum (mouse)</td>
<td>(Stoykova and Gruss, 1994)</td>
</tr>
<tr>
<td>Pax7</td>
<td>Superior colliculus, specific nuclei of the pons/medulla and thalamus; cerebellar Bergmann glia (rat, mouse and chick)</td>
<td>(Shin et al., 2003; Stoykova and Gruss, 1994; Thomas et al., 2007; Thompson et al., 2007; Thompson et al., 2008)</td>
</tr>
</tbody>
</table>
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.** 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
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-Torres 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 transdifferentiation 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 p75NTR (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 (Nebra 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.
FUNDING SOURCES –
During the preparation of this manuscript JT was assisted by grant funding from an Edith Cowan University Industry Collaboration Grant with St John Ambulance (WA) and Lotterywest. MZ was the recipient of grant funding from the NHMRC (Application No 1013349), CaPCREUI and Lotterywest. The funding sources had no involvement in the production of the manuscript.

AUTHOR CONTRIBUTIONS – Both authors participated in the concept of the manuscript. JT wrote the draft manuscript and MZ provided critical review and edited the manuscript. Both authors have approved the final article.

ACKNOWLEDGMENTS
This paper is dedicated to the memory of Mark Kneale, a wonderful brother who taught me the value of “smelling the roses”.

The authors have no competing interests to declare.
REFERENCES


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.


Freed, C. R., Greene, P. E., Breeze, R. E., Tsai, W. Y., DuMouchel, W., Kao, R., Dillon, S., Winfield, H.,


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


Anatomical and functional reconstruction of the nigrostriatal pathway by intranigral


Mangin, J. F., Boisse, M. F., Palfi, S., Cesaro, P., Samson, Y., Hantraye, P., Peschanski, M. and


Progressive loss of PAX9 expression correlates with increasing malignancy of dysplastic and
cancerous epithelium of the human oesophagus. J. Pathol. 197, 293-297.
crest transcription factors distinguish neuroectodermal tumors of varying malignancy and

DNA binding protein expressed during early neurogenesis. EMBO J. 10, 1135-1147.

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.,
of embryonic dopaminergic tissue implants in a subset of transplanted patients with

Development 121, 1433-1442.

Expression of pax6 and sox2 in adult olfactory epithelium. J. Comp. Neurol. 518, 4395-4418.


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.


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.,

Cells and Subsequent Cell Cloning Induced Retinal Neuron Progenitors, Including Retinal


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.

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.
graft survival and striatal reinnervation after the transplantation of fetal mesencephalic


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.


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


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.

Pax6 is required for the multipotent state of retinal progenitor cells. Cell 105, 43-55.


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


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


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.

Ponti, G., Peretto, P. and Bonfanti, L., 2008. Genesis of neuronal and glial progenitors in the

events in thalamocortical tract formation in mice lacking the transcription factors Pax6 or

connections of striatal grafts implanted into the ibotenic acid lesioned neostriatum in adult


78
Shimizu, N., Watanabe, H., Kubota, J., Wu, J., Saito, R., Yokoi, T., Era, T., Iwatsubo, T., Watanabe, T.,

2003. Pax-7 immunoreactivity in the postnatal chicken central nervous system. Anatomical

Sieber-Blum, M., 2010. Epidermal neural crest stem cells and their use in mouse models of spinal

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.


Brain Res. 1208, 25-34.


Ueno, H., Kurokawa, M. S., Kayama, M., Homma, R., Kumagai, Y., Masuda, C., Takada, E., Tsubota, K.,
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.

interaction between p53 and the embryonic proto-oncoprotein PAX3. FEBS Lett. 581, 5831-

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


development of the thymus epithelium and is required for normal T-cell maturation.

Development 122, 23-30.


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.


