

Pharmacological Manipulation of Neural Progenitor Pathways *In Situ*: Possibilities for Neural Restoration in the Injured Adult Brain

Carla B. Mellough¹, Andrew Wood² and Stefan A. Przyborski^{1,*}

¹*School of Biological and Biomedical Science, University of Durham, South Road, Durham DH1 3LE, U.K.*; ²*Wyeth Research, Discovery Neuroscience, Monmouth Junction, Princeton, New Jersey NJ 08852, U.S.A.*

Abstract: Progress over the last decade has confirmed the occurrence of *de novo* neurogenesis within discrete regions of the adult brain. It has been demonstrated that under certain conditions neurogenesis can be stimulated above basal levels in the adult, and that resident pools of adult progenitors can be manipulated to generate new neurons *in situ*. Undoubtedly, these reports prelude possibilities for applications in regenerative medicine. Much attention is now being focused on the elucidation of the discrete mechanisms that are involved in the induction of the neurogenic response in the adult brain and whether these pathways can be pharmacologically manipulated to endogenously replace lost cells and alleviate neuropathy. There is evidence that the re-expression of many key molecular components of the various pathways controlling cellular proliferation, migration and differentiation during development can be re-induced within the mature brain. Recent reports show that the expression of a number of these developmentally-associated molecules occurs in close association with adult progenitor proliferation and neurogenesis, signifying an additional role for these systems in eliciting the adult neurogenic response. Here we review the literature regarding this phenomenon, with reference to the main candidate pathways involved including bone morphogenetic protein, sonic hedgehog and Wnt signalling pathways, and discuss the progress which has been made in the use of small molecules to manipulate these pathways and affect adult neurogenesis *in situ*.

Keywords: Neurogenesis, adult progenitors, Shh, BMPs, Wnt, pharmacological manipulation, endogenous repair.

1. INTRODUCTION

The regenerative capacity of the adult mammalian central nervous system (CNS) is limited and it is generally incapable of replacing functional cells lost in the course of injury or disease. Accordingly, a neurological insult almost always results in permanent functional impairment, and as a consequence, many people remain incapacitated for the remainder of their lives. The personal, clinical and economic implications of this are far reaching, severely affecting the quality of life of millions for people worldwide. The lack of restorative therapies following neural trauma and the significant number of increasingly prevalent neurological diseases emphasises the need for the development of novel approaches which act to allow or induce some recovery of lost or altered function in affected patients.

Recently, much attention has been focused on the support of compromised neural cells or the replacement of lost neural tissues by the delivery of new populations of cells, such as neural precursor cells or stem cells, into injured or degenerate regions of the CNS [1]. Transplanting cells to replenish lost neural populations is especially relevant in the treatment of traumatic, ischemic and degenerative cell loss. In order to achieve appropriate and functional cell replacement within damaged tissues, grafted cells must stably integrate within the correct anatomical regions, survive for extended periods

of time, receive relevant afferents, release appropriate neurotransmitters and reform axonal projections to topographically correct brain regions. However, numerous ethical, immunological and functional issues, remain to be resolved in this process that have considerably slowed the development of neural transplantation therapy [2]. For example, besides the clear ethical considerations associated with the use of embryonic and foetal donor material, it has been reported that a patient suffering from Parkinson's disease will require between 8-12 fetuses for bilateral transplants to be most effective [3]. This is undoubtedly a significant quantity of donor material for success of one transplant and the source of such material is likely to be variable and inconsistent. Furthermore, the results reported in various animal neural transplantation studies have not been reflected in man. Indeed, many clinical neural transplantation trials are inconsistent in their outcome and in some cases cause worsening symptoms and adverse effects [4]. Depending on the source of donor cells, immunological problems can also be prevalent which may result in hyperacute rejection of the xenograft. This, coupled with a gradual decline in the number of successfully-integrated cells over time, results in the loss of a substantial proportion of the initial graft [4-6]. Xenotransplanted cells have been shown to integrate within the mammalian CNS but their long-term functional capabilities remain questionable and their use may risk the introduction of novel diseases in humans.

As research progresses, many of the current obstacles to neural transplantation are likely to be overcome and grafting cells into the CNS may offer a more immediate method to

*Address correspondence to this author at the School of Biological and Biomedical Science, University of Durham, South Road, Durham DH1 3LE, U.K.; Tel: +44 (0)191 3341341; Fax: +44 (0)191 3341201; E-mail: stefan.przyborski@durham.ac.uk

relieving certain neurological deficits. However, replenishing neural circuitry by the transplantation of donor cells within compromised CNS regions is unlikely to provide the principal long-term solution to treating the millions of patients suffering from neurological dysfunction since such neurosurgery will require specialist equipment, expertise and be of significant expense. For this reason, therapeutic transplantation may only be available to the fortunate few. Accordingly, scientists are now exploring a different route to replacing lost cells in the damaged CNS. It is proposed that pharmacological manipulation of existing neural tissues using small molecules or proteins may be a viable approach to encourage the brain to self-repair. In this review, we explore the recent advances in the recruitment of cells from endogenous adult progenitor or stem cell populations as tools for repairing the damaged nervous system and discuss the candidate molecular pathways likely to be targeted by this approach.

2. NEUROGENESIS IN THE ADULT CENTRAL NERVOUS SYSTEM

The classical understanding of neurogenesis in the adult mammalian CNS has recently been re-evaluated in the light of several studies describing the occurrence of *de novo* neurogenesis in discrete regions of the adult brain and the isolation of neural stem cells from these regions [7-20]. It has been shown that stem cells derived from regions of the adult brain including the spinal cord, prefrontal cortex, hippocampus, substantia nigra, ependymal layer of the lateral ventricles, adult ciliary pigmented epithelium and sclera-choroid cells of the eye, can be expanded in culture and differentiate into both neurons and glia *in vitro* or *in vivo* [15, 17, 21-26]. It is suggested that the level of *de novo* neurogenesis has little functional relevance since the number of newly generated cells and discrete regions involved in adult neurogenesis is low, and that there is a lack of spontaneous recovery subsequent to CNS trauma or degenerative disease [27]. There are, however, regions of the CNS where neurogenesis is readily detectable and continues throughout life. In the olfactory bulb, GABA-ergic interneurons are continually replaced by the tangential migration of neuroblasts from the subventricular zone (SVZ), whereas in the subgranular zone (SGZ) of the hippocampal dentate gyrus, neurons and synaptic contacts are constantly remodelled for the purpose of memory-associated plasticity [8, 13, 18, 28-39].

More recently, it has been demonstrated that it may be possible to stimulate adult neurogenesis to generate new neurons *in situ* from resident progenitor cells, even in areas where neurogenesis does not normally occur, and manipulate these precursors *in situ* to selectively replace lost neurons [40-45]. From these studies it appears that adult progenitors are limited only by the microenvironmental neurogenic signals they receive, and that even in the adult, resident progenitors can be manipulated to generate new neurons *in situ*. Transplantation may become unnecessary if we are able to replicate the mechanisms controlling neurogenesis in the adult by prompting the mobilisation and migration of adult progenitors or stem cells towards the site of injury, followed with their appropriate integration within the neural circuitry. If the molecular signals that induce neurogenesis in the adult can be elucidated, then this would have huge implications for the development of pharmaceutical approaches aiming to

improve recovery after CNS trauma or alleviate symptoms of neurodegenerative disease.

3. PATHOLOGY-INDUCED NEUROGENIC CUES IN THE ADULT BRAIN

3.1. Apoptotic Degeneration and Adult Neurogenesis

Inappropriate apoptosis and aberrant activation of caspases underlies the pathology of many human diseases [46] including chronic neurodegenerative conditions such as Parkinson's [47-49], Alzheimer's disease (AD [50-55]), human immunodeficiency virus (HIV) encephalitis [56, 57], epilepsy [58] and amyotrophic lateral sclerosis [59-62] and is implicated in the secondary brain damage seen after stroke [63-67] and traumatic brain injury [68, 69]. Recent results in adult animal models, and observations in the canary over seasonal song modification, suggest that the apoptotic degeneration of endogenous neurons creates a neurogenic-permissive environment for the stable and directed integration of newly transplanted cells, or neurons newly recruited from pools of endogenous neural precursors [40, 41, 70-77]. This appears to involve the expression of specific environmental cues acting to guide cellular migration and phenotypic development within the degenerate region, a result that is not observed in intact hosts or in hosts with necrotic or inflammatory lesions [78]. In the neocortex of adult mice, a typically non-neurogenic region, the selective induction of apoptosis in specific neuronal populations by targeted chromophore photolysis has been shown to induce *de novo* neurogenesis by adult progenitors. These cells can give rise to mature neurons as indicated by retrograde tracing that demonstrated the formation of the appropriate long-distance corticothalamic connections [40]. This method of selective apoptotic depletion has also been shown to support the neuronal differentiation of grafted precursors within the degenerate region [77]. These observations confirm the recapitulation of a neurogenic-permissive microenvironment surrounding non-inflammatory pathology and that the combination of signals are instructive for neural precursors *in situ* as well as exogenously grafted neuronal precursors. The successful integration of endogenous proliferating cells within areas of apoptotic degeneration may depend on sparing of intermixed interneurons, glia, axons or connective tissue and the resultant changes in local microenvironmental signals. Such signals may include the up-regulation of brain derived neurotrophic factor, neurotrophin-4/5 or the trkB receptor, all of which have been observed within regions of non-inflammatory neuronal degeneration [78, 79]. Yet apoptosis does not appear to be the only underlying influence on adult neurogenesis, considering that a neurogenic response can be elicited following seizures in the adult brain which do not cause any discernable cell death [80, 81]. However, the mechanisms surrounding apoptotic degeneration of neurons, do appear to elicit a more direct neuronal-replacement response in the adult when compared with necrotic pathology (see below). This indicates that many neurogenic signals may already exist in brains affected by apoptotic pathology. This is in line with observations made by Jin and co-workers (2004), who recently reported that neurogenesis was up-regulated in patients with Alzheimer's disease and that this coincided with the expression of the cell migration protein, doublecortin, polysialylated nerve cell adhesion molecule

and neurogenic differentiation factor in the hippocampus [82].

3.2. Necrotic Neurodegeneration and Adult Neurogenesis

Necrosis is known to be the primary mode of cell death following inflammation of neural tissues arising from traumatic insults to the brain. Necrosis and the neuro-inflammatory response are generally understood to have an inhibitory effect on adult neurogenesis [83, 84]. However, several studies report the up-regulation of cell proliferation in the brain following various insults, including epileptic seizures [80, 85-87], excitotoxic or mechanical lesions [88, 89] and global forebrain ischemia [90-93]. Until recently, there has been little evidence as to whether such neurogenesis gave rise to surviving neuronal cells that could potentially repopulate necrotically-depleted tissues. Indeed, *de novo* neurogenesis following necrotic pathology appears to generate only a small number of surviving neurons, for example in the dentate gyrus following seizure [80], and in the injured neostriatum in rat stroke models [94, 95]. Levels of neurogenesis following necrosis are generally lower than those observed in the apoptotic brain, and it remains unclear whether new cells arise due to the small apoptotic component of necrotic pathology.

4. ELUCIDATION OF THE MECHANISMS INVOLVED IN ADULT NEUROGENESIS

There has been significant interest in the dissection of the molecular mechanisms involved in the regulation of neurogenesis in the adult brain. Several factors have already been shown to affect the recruitment, migration and maturation of adult neural progenitors, and are associated with neurogenic regions in the adult, or become up-regulated following ischemic or toxic insults. For example, expression of the microtubule binding protein doublecortin is associated with the migration of neuroblasts during development of the CNS and is also found in isolated areas of the adult mammalian brain associated with neurogenesis including the dentate gyrus, the wall of the lateral ventricles and the rostral migratory stream [96-99]. A recent study showed that proliferating progenitor cells and newly generated neuroblasts in the adult mammalian brain are immunopositive for doublecortin and show a sharp decrease in expression upon cellular maturation [100], thereby implicating a role for this protein in adult neurogenesis. Various other markers of cell proliferation can also be found on BrdU-labelled cells in the adult, including proliferating cell nuclear antigen (PCNA), phosphor-histone-H3 and CDC47 [101-103]. Moreover, the secretion of a recently cloned novel secretory factor, Neurogenesis-1, from hippocampal astrocytes and dentate granule cells has been shown to exert a neurogenic effect on adjacent neural stem cells in the adult hippocampus [104]. Clearly some of the prerequisites for neurogenesis remain active within distinct regions of the adult brain [78, 79], which suggests that many of the necessary molecular cues may already be primed and are ready to further stimulate levels of adult neurogenesis.

The definitive combination of factors initiating and controlling neurogenesis in the adult brain remain unclear. Those molecules identified to date include: the mitogens epidermal growth factor, heparin-binding epidermal growth

factor-like growth factor, vascular endothelial growth factor, basic fibroblast growth factor, brain derived neurotrophic factor, insulin like growth factor-1, transforming growth factor- and kit ligand [15, 21, 82, 99, 105-112]; Notch receptor signalling and its ligands Delta and Serrate/Jagged [113]; diffusible factors including nitric oxide and Slit [114-117]; cell cycling genes and transcription factors [118-120]; various surface proteins and substrates including polysialylated-neural cell adhesion molecule and the immature neuronal marker protein TUC-4/CRMP-4/Ulip-1 [121-123]; glutamate activation of N-methyl-D-aspartate or alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors [93, 124, 125]; ageing [16, 126]; physical activity [127]; environmental enrichment and learning [128, 129]; psychosocial stress [125]; oestrogen [130]; testosterone [131]; serotonin [132]; and systemic effects including adrenal steroid levels [133-135] or the administration of antidepressants [136, 137]. Clearly there is an enormous range of variables that appear in some way to influence the activity of endogenous neural progenitor cells in the adult brain. Although several of these effects are mediated by molecules that are known to act via previously described molecular pathways, many of these observations remain difficult to explain since it is unclear by what mechanism(s) some factors mediate neurogenic activity.

There is currently a strong consensus that the molecular control of neurogenic activity in the adult CNS involves many of the regulatory pathways involved in the formation of the CNS during embryonic development. Accordingly, considerable interest is now being directed towards assessing the role of developmentally regulated neurogenic genes in the adult, with particular emphasis on sonic hedgehog (Shh), bone morphogenetic protein (BMP), and Wnt signalling pathways. Several components of these regulatory mechanisms have been identified but it is not yet fully understood how these pathways interact with one another. Therapeutic control of the adult neurogenic responses may lie in understanding the complex interplay between the various components of these pathways shown to influence adult progenitor and stem cell proliferation, migration and differentiation. Studies assessing the affect of the various components of these regulatory mechanisms will allow us to discern the intricate combination of events required to modulate neurogenesis in the adult brain. Furthermore, an understanding of the signals conducive for the control of adult neurogenesis will distinguish whether the mechanisms involved in each step can be harnessed and manipulated as a potential pharmacological approach to encourage the brain to self-repair.

For the sake of simplicity, factors affecting cellular survival become separated at this point – clearly what needs to be elucidated are the discrete mechanisms underlying the neurogenic response itself; the proliferation, cellular migration and appropriate differentiation of adult progenitors and stem cells. Some of the main candidates of the aforementioned pathways and the evidence for their involvement in adult neurogenesis are discussed below.

4.1. Sonic Hedgehog

4.1.1 Role of Sonic Hedgehog in Neural development

The Hedgehog family of secreted proteins has a large number of functions during vertebrate development. Of the

three known mammalian homologues, Sonic hedgehog (Shh) appears to have the most potent effects and has the largest range of biological actions. Shh is a secreted morphogen produced by mid-line cells along the entire neural axis [138]. A large number of studies have shown Shh to be involved in many aspects of CNS development including growth and pattern formation [139-145], cellular proliferation [138, 146-149, 150, 151], telencephalic interneuron development [152], retinal and eye development [153], limb patterning [154] and cell fate determination [150, 155-164].

Sonic hedgehog organises neural tube development by acting as a gradient morphogen, orchestrating the production of homeodomain transcription factors, including Nkx, Pax and Dbx family members, along the dorsoventral axis in a dose-dependent manner [141, 161-164]. These transcription factors ultimately play a role in specifying neural cell type identity and thus Shh is highly involved in neural cell fate determination [161-164]. Shh and BMPs exert opposing actions on proliferation and differentiation during development, and their interaction is crucial for the specification of dorsal and dorsoventral cell types. It is understood that Shh inhibits BMP signalling by activating the BMP inhibitor noggin [165], and acts to enhance oligodendroglial and neuronal differentiation of neural progenitor cells whilst inhibiting the effects of BMP2 on progenitor proliferation and astroglial differentiation [150]. Acting in conjunction with fibroblast growth factor, Shh is also required for the production of dopaminergic and serotonergic neurons in the mid-brain and hindbrain, as well as motorneurons and GABA interneurons in the ventral neural tube [161, 141]. Shh released by Purkinje cells induces the proliferation of embryonic and postnatal cerebellar granule neurons [138, 148]. Shh also regulates the proliferation and survival of cortical astroglia [147] and oligodendrocyte precursors in the spinal cord [146, 151] and is potent mitogen for cultured retinal progenitor cells [159, 160]. Studies in chick and *Drosophila* indicate that cell migration in the embryo also appears to be modulated by Shh [142, 145]. Mice with homozygous null mutations for Shh show absence of ventral cell types in the brain and craniofacial abnormalities including cyclopia [157]. Humans lacking Shh also develop holoprosencephaly and cyclopia due to failure of separation of the forebrain [166]. Shh signalling defects are also associated with basal cell carcinomas and brain cancers including medulloblastomas [167]. Clearly Shh plays a pivotal role in embryonic development and participates in a multitude of important events during the formation of the nervous system.

4.1.2 Mechanism of Sonic Hedgehog Signalling

Research during the last decade has begun to unravel the intricacies of Shh signalling (Fig. 1). Secreted Shh undergoes autocatalytic cleavage into a 19kDa N-terminal (Shh-N) and a 25kDa C-terminal (Shh-C) domain, mediated by the enzymatic activity of Shh-C. The N-terminal peptide is responsible for all of the known signalling functions of the molecule [168]. Shh effects are mediated by the transmembrane molecules patched (Ptc) and smoothened (Smo). Smo does not bind Shh directly, but is activated by interaction with Ptc upon Ptc-Shh-N binding (Fig. 1). Ptc is defined as a tumour-suppressor and silences the pathway in the absence of Hedgehog ligands. Ptc acts as a negative modulator of Shh by op-

posing the actions of Shh by basal repression of Smo. Upon Shh-N binding to Ptc this inhibition is removed and Smo initiates the intracellular Hh signalling cascades which act on the transcription factors of the Gli family and lead to the expression of Shh target genes including Wnt family members and BMPs [169-171]. In addition, Gli1 activity is known to up-regulate platelet-derived growth factor receptor (PDGFR) expression, which has been shown to promote basal cell carcinoma expansion [172], and IGF-2, which is involved in cellular proliferation and implicated in tumorigenesis [173]. The serine/threonine kinase, fused (fu), acts as a positive mediator of Shh signalling, whilst protein kinase A, costal-2 (cos-2), Hedgehog-interacting-protein-1 (Hip1), growth-arrest-specific-1 (GAS-1) and suppressor-of-fused (Sufu) are inhibitors of Shh signalling (Fig. 1) [171, 174, 175]. Proliferative and tumorigenic effects of Shh appear to be mediated by cyclin D1 and the proto-oncogene N-myc, which are shown to be upregulated after Shh activation and are overexpressed in murine medulloblastoma [176, 177]. Recent reports suggest the Shh signalling mechanisms to be more complicated than originally thought, in that an inverse relationship exists between Smo and Ptc. In response to Hh signalling, cell-surface levels of Smo increase whilst levels of Ptc are reduced [178-180]. Furthermore, Shh signalling does not appear to be mediated exclusively by Gli transcription factors, but also by other as yet unidentified factors [181].

4.1.3 Sonic Hedgehog Signalling in the Adult Brain

While significantly less is known about the activity of Shh signalling during neurogenesis in the adult mammalian brain, recent experiments indicate that certain aspects of Shh activity appear to be maintained. For example, the Ptc receptor is expressed in high levels in granule cells of the dentate gyrus and to a lesser extent in the pyramidal cell layers of the hippocampus, suggesting a role for Shh in this actively neurogenic region [182]. Furthermore, isolated adult hippocampal progenitor cells also express Ptc [108]. The proliferative effects of Shh in the developmental context can also be mediated in the adult CNS. For example, adeno-associated viral vector delivery of Shh cDNA in adult rats was shown to produce a 3.3-fold increase in cell proliferation within the hippocampus, with subsequent differentiation of newborn cells into granule neurons [183]. In addition, introduction of cyclopamine (see Fig. 2), a natural inhibitor of Shh, into the adult rat hippocampus was found to reduce cellular proliferation, providing evidence that neuroprogenitor proliferation in the adult brain may be mediated by the Shh signalling pathway [183]. Interestingly, although Shh is expressed in the dentate gyrus of E15 mice, it is not detectable in the adult hippocampus, but it is present in the adult basal forebrain primarily in GABA-ergic neurons that are known to project to the dentate gyrus [182, 184]. In other work, Shh is also shown to be neuroprotective for the nigrostriatal pathway and reduce behavioural impairment in a rat model of Parkinson's Disease [185], whereas the systemic treatment of mice with polyethylene glycol modified Shh enhanced the regeneration of injured sciatic nerves [186]. The activity of Shh may also be appropriate in the treatment of demyelination following spinal cord injury, following a report which showed delivery of Shh into demyelinated lesions in adult rats induced a significant increase

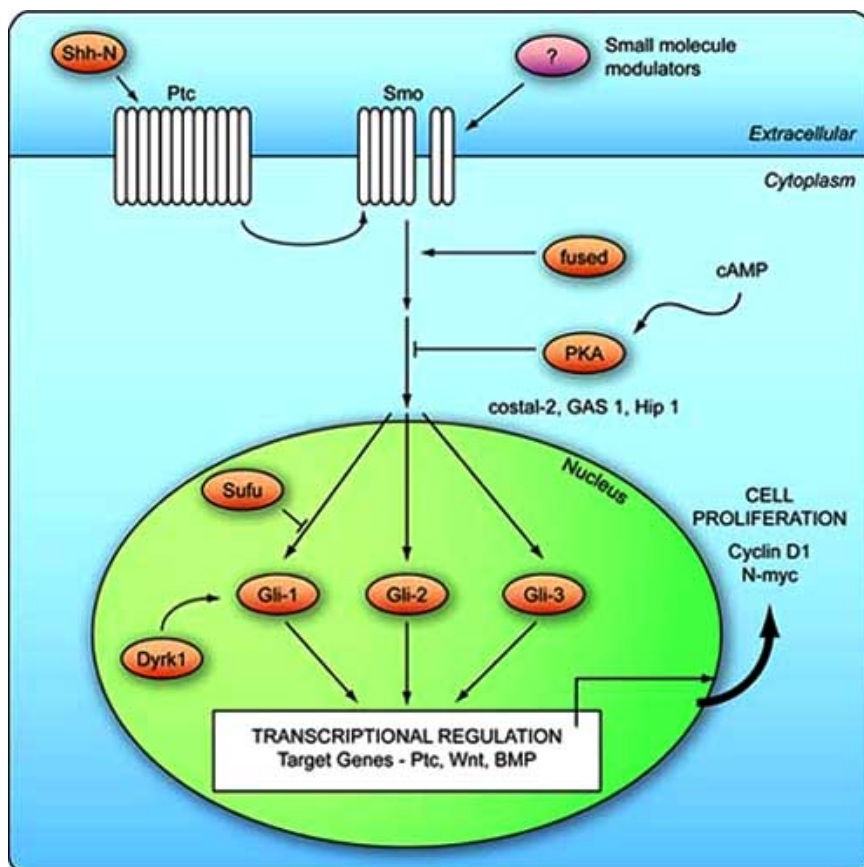


Fig. (1). Mechanisms of the sonic hedgehog (Shh) signalling pathway. Activation of Shh signalling occurs by Shh-N binding to Patched (Ptc), an integral membrane protein with twelve transmembrane domains that acts as an inhibitor of Smoothed (Smo) activation. Upon ligand binding to Ptc, repression of Smo signalling is removed and the Shh intracellular cascade is initiated which acts on members of the Gli family of transcriptional activators. Downstream effects include the induction of Cyclin D1 and N-myc expression, and the transcriptional regulation of Wnt and BMPs. Kinases including PKA and suppressor of fused (Sufu) act at various stages of the Shh pathway to oppose Shh pathway activation, whilst fused and Dyrk1 act to stimulate Shh-induced transcriptional activation. Small molecule agonists and antagonists of the Shh pathway have been identified which act at the level of the Smo receptor. (artwork courtesy of Paul Robinson).

in the number of oligodendrocyte precursors and neurons [187]. Taken together, these reports indicate that Shh signalling appears to function and influence neural cell proliferation and differentiation in the adult brain.

4.1.4 Pharmacological Manipulation of the Sonic Hedgehog Pathway

Researchers are currently investigating the possibility of being able to pharmacologically direct endogenous populations of brain cells to replace lost tissues with the intention to alleviate neurological deficits. The Shh signalling pathway is well suited to such intervention, especially given its known roles in neural differentiation, and that an understanding of some of the molecular mechanisms involved already exists.

Neonatal cerebellar cells cultured *in vitro* in the presence of the biologically active N-terminal fragment of Shh (Shh-N) are shown to display a 100-fold increase in cellular proliferation when compared with controls cultured in the absence of Shh-N [138]. This effect can be observed within 24hrs of Shh exposure and is maintained over long periods in culture, with Shh-treated cells displaying increased proliferation even after 14 days [138]. Granule cell precursors

(GCPs) were identified to be the primary Shh-responsive population within these cultures, whilst astrocyte and oligodendrocyte precursors were found to be unresponsive to the proliferative effects of Shh-N treatment. In cerebellar slice culture systems, addition of Shh-N to the media resulted in the maintenance of proliferative, premigratory GCPs within the EGL and the prevention of withdrawal from the cell cycle and subsequent migration of GCPs from this layer [138]. Forskolin and bFGF were found to exert potent suppression upon Shh-induced proliferation. The transplantation of hybridoma cells which secrete function-blocking anti-Shh-antibodies into the cerebra of P4 mice also substantially inhibited GCP proliferation, and resulted in a substantial decrease in the number of mitotically active cells present in the EGL [138].

Various small molecules have recently been described that might act as agonists or antagonists affecting Hedgehog protein functions. In a screen of compound libraries, Frank-Kamenetsky and colleagues (2002) identified a group of small molecule Hh agonists (Hh-Ag) which interact directly with Smo, thereby overriding the inhibitory effect of Ptc [188]. This class of small molecules are now known as the

leiosamines (Fig. 2) [189]. Low concentrations of these non-peptidyl small compounds are shown to induce the expression of Hh target genes and elicit a similar biological response as native Shh. The activity of leiosamines appear to be dose dependent since these molecules elicit Hedgehog inhibitory responses at higher concentrations [189]. Leiosamines are shown to promote cell type-specific proliferation and concentration dependent differentiation *in vitro* [188]. Interestingly, oral delivery of leiosamines to *Shh*^{-/-} and *Smo*^{-/-} pregnant mice resulted in partial rescue of *Ptc1* expression and midline defects in *Shh*^{-/-} mutant embryos *in vivo*, but had no detectable effect on morphology or *Ptc* expression in *Smo*^{-/-} embryos [188]. These data confirmed that leiosamine activity *in vivo* requires the presence of the Smo receptor.

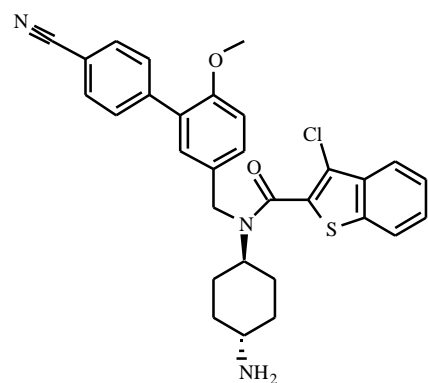
Williams and co-workers (2003) identified a novel small molecule antagonist, Cur61414 (Fig. 2), which acts as an inhibitor of Hh signalling like cyclopamine [190], and also interacts directly with Smo [191]. This compound strongly inhibited Hh signalling in established Hh assays as well as in cells from a *Ptc*-inactive cell line [191]. It is known that Cur61414 competes with leiosamine for binding, thereby

blocking Hh signalling in a *Ptc*-independent manner [189, 191]. Further development of antagonist compounds will provide additional small molecule tools to manipulate endogenous progenitor populations in brain and assess their contribution to the regenerative responses post injury.

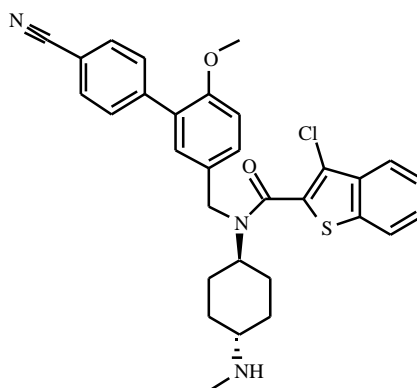
In addition, other small compounds which act similarly to leiosamines and Cur61414 are now being identified [192, 193]. Interestingly, the majority of these synthetic small compounds are understood to interact directly with Smo (Fig. 1) [188, 190, 191]. There is a clear structural homology between the Smo receptor and G protein-coupled receptors (GPCRs), which remain predominately inactive until ligand binding [194]. GPCRs are considered to be tractable drug targets due to their regulatory interactions with small natural ligands, therefore the likeness of Smo to GPCRs may promote this receptor as a promising target for Hh pathway regulation, in the treatment of neurodegenerative disorders [195]. Identification of endogenous ligands may also present opportunities to exploit the properties of natural ligands to modulate this pathway.

(a) Smoothened agonists

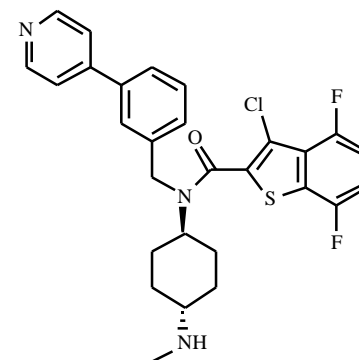
Leiosamines



Hh-Ag 1.1

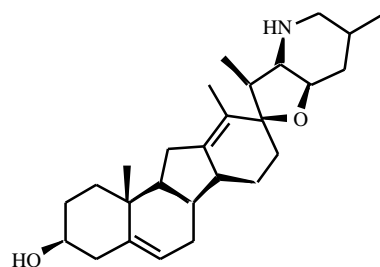


Hh-Ag 1.2

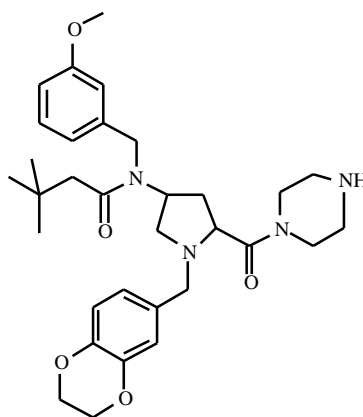


Hh-Ag 1.5

(b) Smoothened antagonists



Cyclopamine



Cur61414

Fig. (2). The molecular structure of some identified natural and synthetic modulators of the Hedgehog (Hh) pathway. **(a)** Smoothened agonists: The leiosamines, comprising the small molecule modulators Hh-Ag 1.1, 1.2 and 1.5 [188]. **(b)** Smoothened antagonists: Cyclopamine, a natural inhibitor of Hh signalling [190], and the synthetic small molecule Cur61414 [191]. See text for further details.

4.2. Bone Morphogenetic Proteins

4.2.1 Role of Bone Morphogenetic Proteins Neural Development

Bone morphogenetic proteins (BMPs) are cytokines and members of the TGF- β superfamily. They were originally identified as proteins capable of inducing ectopic bone formation in rats [196, 197] and independently as dorsalising agents involved in neural fate determination [198-201]. BMPs and their receptors have complex regulatory roles in neurogenesis. Cells respond in a differential manner to BMPs that appears to be related to the cell type and the status of their ontogeny [202]. For example, BMP4 acts as the major dorsalising signal during neural tube formation, whereas both BMP2 and 4 regulate the fate decisions of neural crest cells [203]. Both BMP2 and BMP4 play a role in the formation of the autonomic nervous system and have been shown to induce expression of MASH1, a basic helix-loop-helix transcription factor essential for autonomic neurogenesis [204]. During the period of perinatal cortical gliogenesis, BMPs lead to the cessation of cell proliferation and induction of astrocyte differentiation in cultured progenitor cells from the subventricular zone surrounding the lateral ventricles adjacent to the striatum [150, 205]. In contrast, BMPs appear to function and inhibit oligodendroglialogenesis at all stages

during development [202]. Cultured embryonic neural progenitor cells synthesise and release BMPs, and addition of the BMP inhibitor, noggin, to these cultures enhances oligodendroglial differentiation [206]. Depending on the developmental context, Shh can act to upregulate BMPs or inhibit them by inducing noggin [165, 207]. Similarly, fibroblast growth factor, a known mitogen, has also been shown to antagonise BMP signalling [208]. BMPs are also shown to induce the differentiation of neuronal precursors in the spinal cord, GABAergic neurons and oligodendrocytes in the cortex, and cultures of neocortical precursors from the ventricular zone [209-211]. In cell culture systems, BMPs have been shown to act directly on regulators of cyclin dependant kinases, such as p21^{CIP1} to induce apoptosis or cell cycle exit [212]. This study suggests that BMPs also play a pivotal role in regulating the cell cycle and progressive neural fate decisions in various progenitor cell populations.

4.2.2 Mechanism of Bone Morphogenetic Signalling

BMPs are synthesised as large precursor proteins that undergo proteolytic cleavage to generate C-terminus biologically active BMP dimers [213, 214]. BMP signalling is activated by binding of the BMP ligand to transmembrane serine threonine kinase receptors (Fig. 3). There are various types of BMP receptors (BMPR), including BMPR-1A, BMPR-1B

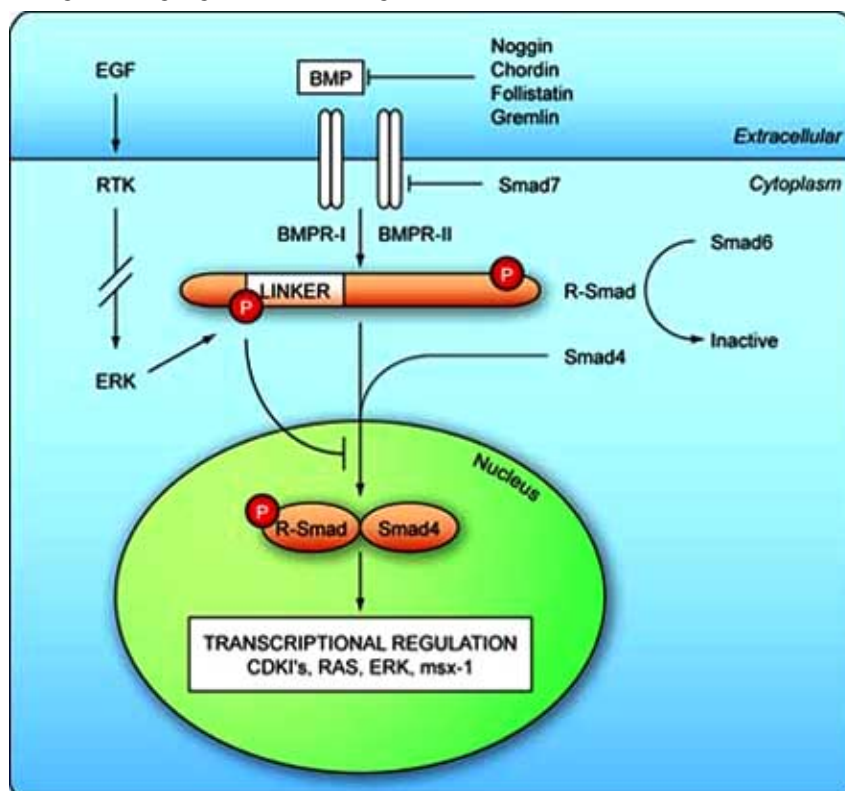


Fig. (3). Mechanisms of the bone morphogenetic protein (BMP) pathway. The binding of BMP dimers to the BMPR causes transphosphorylation of receptor subunits resulting in the recruitment and phosphorylation of the pathway restricted Smads (R-Smad). R-Smad then forms a complex with common mediator Smad (Smad4). The Smad1-Smad4 complex translocates to the nucleus and initiates transcription of target genes, including CDKIs and msx-1. A number of regulatory mechanisms exist at various stages of the BMP pathway. Extracellular proteins including Noggin and Chordin act to antagonise BMP signalling by binding BMP and competing at the BMPR. Within the cell, Smads 6 and 7 repress BMP pathway activation in the cytoplasm and negative feedback exists simultaneously at the nuclear level. (artwork courtesy of Paul Robinson).

and BMPR-II, all of which are expressed abundantly throughout embryogenesis and adult life [215-217]. There are three known Type-I and three known Type-II BMPR subtypes, combinations of which form a tetrameric receptor complex [218]. Both receptor subtypes are required for pathway activation [219-221]. Ligand binding leads to heteromeric receptor complex formation, BMPR subtype-I phosphorylation, and activation of the intracellular signalling cascade [221-223]. BMP signalling involves a complicated array of negative feedback loops at various stages along the BMP pathway. A number of structurally distinct proteins in the extracellular space specifically bind BMP thereby blocking ligand activity, and which also compete with BMP at the receptor level (Fig. 3) [219, 224-226]. These antagonists, which include Gremlin, Noggin, Chordin and follistatin, act to regulate BMP cascade initiation [227]. BMP ligands bind in a cooperative manner to specific receptor subunits resulting in transphosphorylation of the type-I subunit by the type-II receptor resulting in the propagation of the intracellular cascade [220, 221]. Activation of the BMPR-I subunit results in the recruitment and phosphorylation of the latent pathway restricted Smads (R-Smads 1, 5 or 8). Upon phosphorylation, activated R-Smad is released from the receptor into the cytoplasm then recruits and forms a complex with common mediator Smad4 (Fig. 3). In the intracellular compartment this signal is modulated by the activation of inhibitory Smad proteins, Smad6 and Smad7. The Smad1-Smad4 complex translocates to the nucleus to initiate gene transcription of specific target genes, affecting members of the cyclin dependent kinase inhibitory (CDKI) genes including p15, p21 and p38 [228] *msx-1*, *RAS* and *ERK* [229]. Negative feedback also exists at the nuclear level, where gene transcription requires the presence of coactivators and can be inhibited by co-repressors [229].

4.2.3 Bone Morphogenetic Protein Signalling in the Adult Brain

A number of BMPs and their receptors are expressed in perinatal tissues including the hippocampus, cortex and cerebellum. BMP6 is highly expressed in the brain at all stages of development and also in the adult hippocampus and neocortex [230, 231]. BMP6 can bind to each of the BMP receptor types, however BMPR-II is present in the adult cortex, dentate, hippocampus and substantia nigra [215], and becomes upregulated in the granule cells of the dentate gyrus and after transient global cerebral ischemia and brain contusion [232, 233]. The function of BMP6 in the brain appears to be dose-dependent. Pre-treatment with low concentrations of BMP6 (1.25×10^{-9} mol/L) has been shown to increase motor performance and decrease cerebral infarction caused by arterial occlusion in adult rats by reducing caspase-3 activity and DNA fragmentation [234]. Interestingly, at higher concentrations, 12.5×10^{-9} mol/L BMP6 induces granule cell differentiation suggesting this protein could play multiple roles when used in a therapeutic context [206, 235]. BMP7 (known as osteogenic protein-1 or OP1) is also found in the adult hippocampus, cortex and cerebellum and binds to BMPR-II. BMP7 has been shown to reduce infarction volume and mortality in neonatal and adult rats [236, 237] and appears to enhance sensorimotor recovery following focal stroke by promoting dendritic sprouting and neurite outgrowth [238-240]. BMP7 can also stimulate BrdU incorpo-

ration into glial cells, increasing proliferation of immature glial cells and astrocyte numbers *in vitro* [240]. This is extremely interesting considering growing evidence suggesting that GFAP-positive astrocytes in the adult brain give rise to new neurons in the hippocampus and therefore astroglia may be the source of the adult neural stem cell niche [241, 242]. Indeed, if this is the case, BMP7 could play a major role in the control of adult neurogenesis by encouraging the proliferation of these adult stem cell-like astroglial cells.

4.2.4 Pharmacological Manipulation of the Bone Morphogenetic Pathway

To the best of our knowledge, no literature exists which describes the development of small molecule agonists or antagonists of the BMP pathway relating to the brain. There are, however, a number of studies describing the use of synthetic molecules which act via the BMP pathway to modulate bone formation and tumour progression [243-251]. These molecules provide a starting point for the development of neural modulators of BMP signalling and offer important information on aspects of BMP pathway activation.

BMPs are known potent inducers of bone formation. The synthetic anti-angiogenic agent TNP-470 [243-246], an analog of the naturally secreted fumagillin, is shown to strongly inhibit angiogenesis and suppress tumour growth in chick and rat assays [243, 245]. TNP-470 is also shown to reduce BMP-2-induced ectopic bone formation in mice, by preventing the proliferation of mesenchymal cells and chondrogenesis [249]. The action of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) to lower serum cholesterol are well documented and they are used successfully to improve cardiovascular health [252]. Interestingly, the stimulatory effects of statins on bone formation *in vitro* and *in vivo* have also been reported [250, 253, 254]. This work describes the positive effect of statins on bone formation when delivered locally to bony sites and following systemic administration. It is unclear exactly how statins induce bone formation, but it is thought to occur via stimulation of BMP-2 [254].

4.3. Wnt - - Catenin Pathway

The Wnt genes encode a family of cysteine-rich proteins that are vital intercellular signalling molecules during development. The proteins and molecules of the Wnt pathway are critical for normal brain development and are involved in multiple aspects of neurogenesis including dendritic morphogenesis and synapse formation [170]. The Wnt pathway is also implicated in cancer pathology as well as neurodevelopmental, psychiatric, and neurologic disease [255]. -catenin, initially discovered as a cytoskeletal protein, is a major downstream transcription factor in the Wnt pathway and has a role in neuronal differentiation and dendritic morphogenesis [256].

4.3.2 Mechanism of Wnt- -Catenin Signalling

The Wnt pathway receptor, frizzled, is characterised by a N-terminal signal peptide, a cysteine-rich ligand-binding domain (CRD) followed by a hydrophilic linker, seven transmembrane regions, and a cytoplasmic tail [257]. Binding of Wnt ligands to frizzled receptors activates the Wnt signalling cascade and the downstream molecule dishevelled

(*dsh*), thereby inhibiting the kinase GSK-3 (Fig. 4). In basal conditions, GSK-3 phosphorylates β -catenin causing its degradation. Upon inhibition of GSK-3 by *dsh* activation, active β -catenin accumulates in the cytosol. Active cytosolic β -catenin translocates to the nucleus and acts as a transcriptional activator resulting in gene transcription at Lef1/TCF sites [258]. Nuclear β -catenin appears to induce gene transcription to activate the cell cycle and therefore maintain stem cells in an undifferentiated state by forcing cells to re-enter the cell cycle [256, 259]. β -catenin also serves as a mediator between actin and membrane-spanning cadherins, thereby playing a role in the production and maintenance of synaptic connections, and functions to recruit scaffolding molecules towards developing synapses [260].

Frizzled-related proteins (FRPs) are secreted proteins with a highly conserved cysteine residue similar to Frizzleds. They contain a region with similarity to netrins, secreted proteins involved in axon guidance. FRPs can bind directly to Wnt and thereby act as secreted Wnt antagonists (Fig. 4) [261, 262]. It is presently unclear how this process is regulated, but is intriguing that this pathway remains active during cell division, proliferation and in the formation of synaptic connections [260, 263-265]. Mutations in Wnt 3a lead to agenesis of the hippocampus, similar to the effects of the

genetic deletion of Lef1 [255, 266, 267]. Abnormal social and neurological defects are seen in mice lacking the mouse homologue of *dsh*, although these animals are anatomically normal and fertile [268]. Neonatal overexpression of GSK-3 results in reduced brain size in mice [269] and inactivation of the β -catenin gene is associated with brain malformation and abnormalities [270]. Wnt pathway dysfunction is also implicated in the pathophysiology of Alzheimer's disease [271].

4.3.3 Wnt- β -Catenin Signalling in the Adult Brain

Interestingly, mood stabilisers, antidepressants and extreme mood altering therapies have been reported to exert neurotrophic and neurogenic effects. Recent data show that, in experimental adult models, a robust increase in hippocampal neurogenesis follows ECT treatment [81, 137]. Lithium treatments of severe depression, previously shown to increase the levels of Bcl-2 [272], also results in significant increases (25%) in the number of BrdU-labelled cells in the dentate gyrus in chronically lithium-treated adult mice [136]. Small molecule antidepressants are also shown to up-regulate adult neurogenesis, although not to the same extent as ECT. This increase in neurogenesis appears to be mediated, in part by the Wnt - β -catenin pathway.

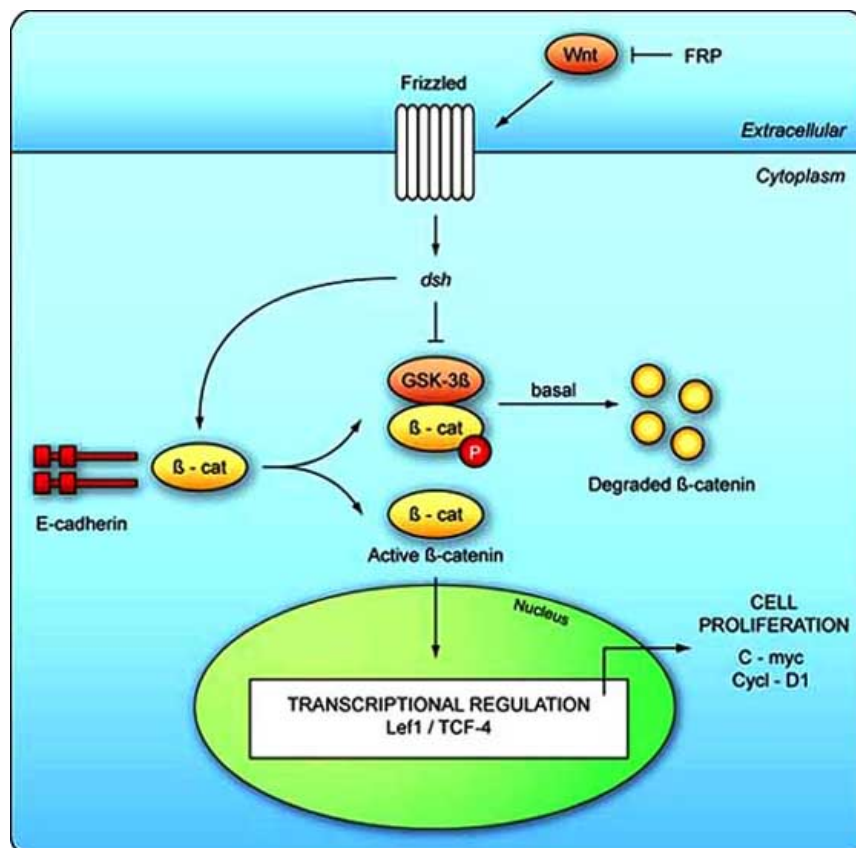


Fig. (4). Mechanisms of the Wnt - β -catenin pathway. In basal conditions, the kinase GSK-3 phosphorylates β -catenin, causing its degradation. Extracellular FRPs antagonise Wnt signalling by binding directly to Wnt. Upon binding of Wnt ligands to the Frizzled receptor, the downstream molecule *dsh* is activated which inhibits GSK-3 activity and results in the accumulation of active β -catenin in the cytosol. Active β -catenin then translocates to the nucleus and activates gene transcription at Lef1/TCF-4 sites to activate the cell cycle. Active cytosolic β -catenin also functions to recruit scaffolding molecules, such as cadherins, that contribute toward the development of synapses. (artwork courtesy of Paul Robinson).

It has been shown that chronic ECT leads to up-regulation of Wnt-2 expression in the hippocampus and levels of β -catenin immunoreactivity in the proliferative zone of the adult hippocampus. The increase in β -catenin seems to occur in close correspondence with cells that are proliferating or have recently undergone cell division [255]. A recent study also demonstrates that blockade of neurogenesis results in suppression of the action of antidepressant treatment in behavioural models of depression [273], providing support for a functional role of this process in the action of antidepressant treatment. It has recently been reported that GSK-3 is inhibited by lithium, resulting in the accumulation of active β -catenin [274]. This kinase is also shown to display mood-stabilising drug-induced axon growth and synaptic remodelling [275] and to modulate BDNF-signalling *in vitro* [276]. Many of the mechanisms of Wnt signalling remain to be elucidated, however the role that the Wnt- β -catenin pathway plays in neurogenesis and dendritic and synapse formation makes components of this pathway ideal targets for manipulating adult neurogenesis.

4.3.4 Pharmacological Manipulation of the Wnt- β -Catenin Pathway

Glycogen synthase kinase-3 (GSK3) is a key component of the Wnt signalling cascade and is associated with neuronal apoptosis following stroke or head trauma, and the hyperphosphorylation of tau protein implicated in the pathophysiology of Alzheimer's disease. A number of potent GSK3 inhibitors have been identified which are shown to modulate glycogen metabolism and gene transcription [277].

Fishman and others (2002) report that activation of the A3 adenosine receptor (A3AR) in melanoma cells by the synthetic adenosine analog IB-MECA, results in an increase in GSK3 and a substantial decrease in the levels of phosphorylated GSK3 and β -catenin [278, 279]. Furthermore, the level of growth regulatory genes cyclin D1 and c-myc were severely reduced following application of IB-MECA [278]. *In vivo*, A3AR agonists are shown to be neuroprotective following ischemia and also suppress tumour growth via de-regulation of the Wnt signalling pathway [279].

Although lithium treatment is shown to increase hippocampal neurogenesis [280, 272], presently there are no reported synthetic small molecules that act comparably to lithium, GSK3 inhibitors or IB-MECA in brain tissue. However, the potent effects of these molecules on the Wnt pathway identify the Wnt signalling cascade as a promising target for neural therapy, particularly related to stroke, head trauma and Alzheimer's disease.

5. CONCLUSION

To date, neurorestorative small molecule or protein therapies have largely been designed as neurotrophic stimulators of established neuronal architecture, seeking to increase cell survival, axon growth or synaptic connectivity. Notably, Cortex, Guilford Pharmaceuticals, Boston Life Sciences, Spectrum Pharmaceuticals, Eli Lilly and Vertex Pharmaceuticals all have active or recent drug development efforts. A smaller set of companies including Curis, Inc., Juventis, Memory Pharmaceuticals, and NeuroNova are actively pursuing neurogenesis as a therapeutic target.

Before the factors known to control neurogenesis can be utilised for therapeutic purposes, it is critical that we first understand the mechanisms responsible for the directed proliferation, differentiation and migration of the various neuronal cell types under the specific conditions of each pathology [281, 282]. Studies of neural development suggest that the biological steps leading to neurogenesis occur by step-wise restriction and are dependent on available microenvironmental signals [283]. These steps are different at various stages of development and in different locations of the brain, highlighting the importance of realising and investigating the regional variance in response to neurogenic factors. For example, infusion of epidermal growth factor into the ventricle of adult rats triggers neurogenesis in the subventricular zone, but it is not effective in eliciting the same response in the subgranular zone of the hippocampus [107, 284]. Furthermore, immature granule cells in the adult dentate gyrus cannot be antagonised by gamma-aminobutyric acid, in contrast to the inhibitory effects this neurotransmitter has on mature granule neurons [285, 286]. The effects of mitogens on cell proliferation also differs during development, for example, early neural stem cells proliferate in response to fibroblast growth factor [287, 288], later appearing stem cells to fibroblast growth factor or epidermal growth factor [289, 290], and more restricted precursors require different conditions again. Glial precursors respond to platelet-derived growth factor and fibroblast growth factor [267], whereas neuronal precursors respond to Shh, fibroblast growth factor and neurotrophin-3 [22].

Knowing the correct combinations of compounds to employ during each stage of adult neurogenesis, and the right order in which to administer them, is extremely important if pharmacological manipulation of endogenous cells is to be successful. Depending on the pathology, different combinations of compounds could be used to achieve the most desired effects. For example, Shh levels could be fine tuned to increase oligodendroglialogenesis and induce remyelination of peripheral and central nerves in multiple sclerosis patients, or encourage dopaminergic lineage induction in the brain of Parkinson's disease patients. Along each step of the way these compounds must also be present in the correct physiological amounts. Low concentrations of particular neurogenic molecules may possibly induce opposing effects to high concentrations of the same compound, as many of the effects of pathway components are dose-dependent. Mis-expression of Shh may elicit abnormal proliferation and supernumerary oligodendrocytes [143, 291] and loss of Shh signalling induces apoptosis of subventricular zone cells [292]. Of greater concern, however, is up-regulation or mis-expression of the proto-oncogene Smo or de-regulation of the Shh signal-transduction pathway since this may result in the induction of basal cell carcinomas or medulloblastomas by instructing abnormal hyperproliferation of precursors. Furthermore, members of the Gli family and insulin-like growth factor signalling are implicated in tumorigenesis [173, 195, 293, 294], and activation of the fibroblast growth factor and Wnt pathways is reported to induce mammary tumours in mice [295, 296]. The effects of using combinations of agonists/antagonists to manipulate different molecular pathways must also be elucidated. For example, the Shh inhibitor GAS-1 is also an inducible gene of the Wnt

pathway, a further important regulatory mechanism of cellular differentiation, and is expressed in regions responsive to Shh [297]. Accordingly, regular screening for abnormal pathway function will clearly be of the utmost importance.

Small, soluble molecule agonists and antagonists are likely to hold most promise for modulation of neurogenesis in the adult brain, however the delivery of therapeutic compounds must be carefully considered. Targeted delivery of reagents to the CNS may be required and systemic administration avoided, given the large range of effects that Shh, BMPs and other molecules can induce throughout the body during the life of an organism. As mentioned previously, the action of Shh is not exclusively targeted toward neural tissue since it can also induce the proliferation of pluripotent blood cell precursors [298]. Thus systemic administration of Shh may induce detrimental effects in the haematopoietic system.

In conclusion, despite the promising advances in this highly relevant field, it is essential that the limitations of exploiting endogenous neural stem cells are resolved before this approach moves into the clinic as a therapy for restoration of the injured adult brain. It is possible that, in some discrete areas of the brain, transplantation might remain the only option for neural replenishment if a substantial neurogenic response cannot be produced. However, it is hopeful, that the development of specifically targeted compounds will allow pharmacological intervention in the adult brain to modulate the molecular mechanisms that control neurogenesis and aid patient recovery from traumatic or neurodegenerative pathologies. Indeed, as molecular pathways become elucidated new targets will become identified providing opportunities for small molecule screening. As in the case of the hedgehog pathway, development of a robust readout enabling a small molecule high throughput screen has provided access to novel molecular entities modulating the pathway and the potential for drug development and tool compounds to further dissect the functional role of hedgehog signalling. Small molecule screening platforms may use reporter systems proximal within the signal transduction cascade or functional readouts with follow up to elucidate the target of compound activity. The challenges to identifying the most tractable targets to modify endogenous stem cell populations are best met by the marriage of pharmaceutical technologies and a clear understanding of the molecular pathways controlling stem cell behaviour under normal and disease conditions. Recently, Curis, Inc. have partnered their BMP portfolio with Ortho Biotech a subsidiary of Johnson & Johnson and their hedgehog proteins and novel small molecule hedgehog pathway agonists with Wyeth Pharmaceuticals, both for neurological disorders. The neurodegenerative diseases that potential stem cell therapies seek to address continue to experience high unmet need and this fact alone will continue to be a driver to foster academic and industrial partnerships.

ABBREVIATIONS

AD	= Alzheimer's disease
BMP	= Bone morphogenetic protein
BMPR	= BMP receptor
BrdU	= 5-Bromo-2-deoxyuridine

CDC47	= Saccharomyces cerevisiae mini-chromosome maintenance protein 7
cDNA	= Cytokine Deoxyribose Nucleic Acid
CNS	= Central nervous system
dsh	= Dishevelled
ECT	= Electroconvulsive therapy
FRP	= Frizzles-related protein
GABA	= Gamma-aminobutyric acid
GCPs	= Granule cell precursors
GFAP	= Glial fibrillary acidic protein
GPCRs	= G protein-coupled receptors
Hh	= Hedgehog
HIV	= Human immunodeficiency virus
PCNA	= Proliferating cell nuclear antigen
Ptc	= Patched
SGZ	= Subgranular zone
Shh	= Sonic hedgehog
Smo	= Smoothed
Sufu	= Suppressor of fused
SVZ	= Subventricular zone
TGF	= Transforming growth factor
trkB	= Tyrosine kinase receptor

REFERENCES

- [1] Bjorklund, A., Gage, F.H. *Ann. N.Y. Acad. Sci.*, **1985**, 457, 53-82.
- [2] Stein, D.G. *J. Neurosurg. Anesthesiol.*, **1991**, 3(3), 170-89.
- [3] Barker, R.A., Dunnett, S.B., Richards, A. *Transplantation.*, **1999**, 68(8), 1091-1092.
- [4] 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., Fahn, S. *New Eng. J. Med.*, **2001**, 344(10), 710-719.
- [5] Barker, R.A., Dunnett, S.B., Faissner, A., Fawcett, J.W. *Exp. Neurol.*, **1996**, 141, 79-93.
- [6] Clarkson, E.D., Zawada, W.M., Bell, K.P., Esplen, J.E., Choi, P.K., Heidenreich, K.A., Freed, C.R. *Exp. Neurol.*, **2001**, 168, 183-191.
- [7] Altman, J. *Science*, **1962**, 135, 1127-1128.
- [8] Altman, J., Das, G.D. *J. Comp. Neurol.*, **1965**, 124(3), 319-335.
- [9] Goldman, S.A. Nottebohm, F. *Proc. Natl. Acad. Sci. USA*, **1983**, 80(8), 2390-2394.
- [10] Kaplan, M.S., Bell, D.H. *J. Neurosci.*, **1984**, 4(6), 1429-1441.
- [11] Richards, L.J., Kilpatrick, T.J., Bartlett, P.F. *Proc. Natl. Acad. Sci. USA*, **1992**, 89, 8591-8595.
- [12] Cameron, H.A., Woolley, C.S., McEwen, B.S., Gould, E. *Neurosci.*, **1993**, 56(2), 337-344.
- [13] Luskin, M.B. *Neuron*, **1993**, 11, 173-189.
- [14] Lois, C., Alvarez-Buylla, A. *Proc. Natl. Acad. Sci. USA*, **1993**, 90(5), 2074-2077.
- [15] Reynolds, B.A., Weiss, S. *Science.*, **1992**, 255(5052), 1707-1710.
- [16] Kuhn, H.G., Dickinson-Anson, H., Gage, F.H. *J. Neurosci.*, **1996**, 16(6):2027-2033.
- [17] Weiss, S., Dunne, C., Hewson, J., Wohl, C., Wheatley, M., Peterson, A.C., Reynolds, B.A. *J. Neurosci.*, **1996**, 16(23), 7599-609.
- [18] Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A. *Nat. Med.*, **1998**, 4(11), 1313-7.
- [19] Gross, C.G. *Nat. Rev. Neurosci.*, **2000**, 1, 67-73.
- [20] Gage, F.H. *J. Neurosci.*, **2002**, 22(3), 612-613.
- [21] Ray, J., Peterson, D.A., Schinstine, M., Gage, F.H. *Proc. Natl. Acad. Sci. USA*, **1993**, 90(8), 3602-6.

- [22] Sensenbrenner, M., Deloulme, J.C., Gensburger, C. *Rev. Neurosci.*, **1994**, 5(1), 43-53.
- [23] Rietze, R.L., Valcanis, H., Brooker, G.F., Thomas, G., Voss, A.K., Bartlett, P.K. *Nature*, **2001**, 412(6848), 736-739.
- [24] Song, H.J., Stevens, C.F., Gage, F.H. *Nature Neurosci.*, **2002**, 5(5), 438-445.
- [25] Lie, D.C., Dziejczapolski, G., Willhoite, A.R., Kaspar, B.K., Shults, C.W., Gage, F.H. *J. Neurosci.*, **2002**, 22(15), 6639-6649.
- [26] Arsenijevic, Y., Taverney, N., Kostic, C., Tekaya, M., Riva, F., Zografos, L., Schorderet, D. *Invest. Ophthalmol. Vis. Sci.*, **2003**, 44(2), 799-807.
- [27] Nakatomi, H., Kuriu, T., Okabe, S., Yamamoto, S., Hatano, O., Kawahara, N., Tamura, A., Kirino, T., Nakafuku, M. *Cell*, **2002**, 110(4), 429-41.
- [28] Altman, J., Das, G.D. *J. Comp. Neurol.*, **1966**, 126, 337-389.
- [29] Hinds, J.W. *J. Comp. Neurol.*, **1968**, 134, 305-322.
- [30] Altman, J. *J. Comp. Neurol.*, **1969**, 137, 433-458.
- [31] Kaplan, M.S., Hinds, J.W. *Science*, **1977**, 197, 1092-1094.
- [32] Kishi, K. *J. Comp. Neurol.*, **1987**, 258, 112-124.
- [33] Lois, C., Alvarez-Buylla, A. *Science*, **1994**, 264, 1145-1148.
- [34] Rousselot, P., Lois, C., Alvarez-Buylla, A. *J. Comp. Neurol.*, **1995**, 351, 51-61.
- [35] Garcia-Verdugo, J.M., Doetsch, F., Wichterle, H., Lim, D.A., Alvarez-Buylla, A. *J. Neurobiol.*, **1998**, 36, 234-248.
- [36] Gould, E., Reeves, A.J., Fallah, M., Tanapat, P., Gross, C.G., Fuchs, E. *Proc. Natl. Acad. Sci. USA*, **1999b**, 96, 5263-5267.
- [37] Gould, E., Tanapat, P. *Neurosci.*, **1997**, 80, 427-436.
- [38] Kornack, D.R., Rakic, P. *Proc. Natl. Acad. Sci. USA*, **1999**, 96(10), 5768-73.
- [39] Kornack, D.R., Rakic, P. *Science*, **2001**, 294(5549), 2127-30.
- [40] Belluzzi, O., Benedusi, M., Ackman, J., LoTurco, J.J. *J. Neurosci.*, **2003**, 23(32), 10411-8.
- [41] Magavi, S.S., Leavitt, B.R., Macklis, J.D. *Letters to Nature*, **2000**, 405(6789), 951-5.
- [42] Scharff, C., Kim, J.R., Grossman, M., Macklis, J.D., Nottebohm, F. *Neuron*, **2000**, 25(2), 481-92.
- [43] Magavi, S.S., Macklis, J.D. *Dev. Brain Res.*, **2002**, 134, 57-76.
- [44] Arlotta, P., Magavi, S.S., Macklis, J.D. *Exp. Gerontol.*, **2003**, 38, 173-182.
- [45] Agasse, F., Roger, M., Coronas, V. *Eur. J. Neurosci.*, **2004**, 19(6), 1459-68.
- [46] Mattson, M.P. *Nat. Rev. Molec. Cell Biol.*, **2000**, 1(2), 120-129.
- [47] Michel, P.P., Hirsch, E.C., Agid, Y. *Rev. Neurol. (Paris)*, **2002**, 158(122), 24-32.
- [48] Tatton, W.G., Chalmers-Redman, R., Brown, D., Tatton, N. *Annals Neurol.*, **2003**, 53(3 Suppl. 1), S61-72.
- [49] Lev, N., Melamed, E., Offen, D. *Prog. Neuropsych. Biol. Psych.*, **2003**, 27(2), 245-50.
- [50] Smale, G., Nichols, N.R., Brady, D.R., Finch, C.E., Horton, W.E. Jr. *Exp. Neurol.*, **1995**, 133, 225-30.
- [51] Wolozin, B., Iwasaki, K., Vito, P., Ganjei, K., Lacaná, E., Sunderland, T., Zhao, B., Kusiak, J.W., Wasco, W., D'Adamio, L. *Science*, **1996**, 274, 1710-13.
- [52] Vito, P., Lacaná, E., D'Adamio, L. *Science*, **1996**, 271, 521-15.
- [53] Anderson, A.J., SU, J.H., Cotman, C.W. *J. Neurosci.*, **1996**, 16, 1710-1719.
- [54] Passer, B., Pellegrini, L., Russo, C., Siegel, R.M., Lenardo, M.J., Schettini, G., Bachmann, M., Tabaton, M., D'Adamio, L. *J. Alzheimers Dis.*, **2000**, 2(3, 4), 289-301.
- [55] Marx, J. *Science*, **2001**, 293(5538), 2192-2194.
- [56] Petit, C.K., Roberts, B. *Am. J. Pathol.*, **1995**, 146, 1121-1130.
- [57] Bartz, S.R., Emerman, M. *J. Virol.*, **1999**, 73, 1956-1963.
- [58] Roux, P.P., Colicos, M.A., Barker, P.A., Kennedy, T.E. *J. Neurosci.*, **1999**, 19(16), 6887-6896.
- [59] Troost, D., Aten, J., Morsink, F., de Jong, J.M. *Neuropathol. Appl. Neurobiol.*, **1995**, 21, 498-504.
- [60] Cookson, M.R., Shaw, P.J. *Brain Pathol.*, **1999**, 9, 165-186.
- [61] Guégan, C., Przedborski, S. *J. Clin. Invest.*, **2003**, 111(2), 153-161.
- [62] Friedlander, R.M. *New Eng. J. Med.*, **2003**, 348, 1365-1375.
- [63] Ni, B., Wu, X., Su, Y., *J. Cereb. Blood Flow Metab.*, **1998**, 18, 248-256.
- [64] Namura, S., Zhu, J., Fink, K. *J. Neurosci.*, **1998**, 18, 3659-3668.
- [65] Hara, A., Yoshimi, N., Hirose, Y. *Brain Res.*, **1995**, 697, 247-250.
- [66] Nitatori, T., Sato, N., Waguri, S., Karasawa, Y., Arraki, H., Shibananai, K., Kominami, E., Uchiyama, Y. *J. Neurosci.*, **1995**, 15, 1001-1011.
- [67] Schulz, J.B., Weller, M., Moskowitz, M.A. *Annals of Neurol.*, **1999**, 45, 421-429.
- [68] Yakovlev, A.G., Knoblach, S.M., Fan, L. *J. Neurosci.*, **1997**, 17, 7415-7424.
- [69] Bengzon, J., Mohapel, P., Ekdahl, C.T., Lindvall, O. *Prog. Brain Res.*, **2002**, 135, 111-9.
- [70] Kim, J., O'Loughlin, B., Kasparian, S., Nottebohm, F. *Proc. Natl. Acad. Sci. USA*, **1994**, 91, 7844-7848.
- [71] Fricker-Gates, R., Shin, J.J., Tai, C.C., Catapano, L.A., Macklis, J.D. *J. Neurosci.*, **2002**, 22(10), 4045-56.
- [72] Shin, J.J., Fricker-Gates, R.A., Perez, F.A., Leavitt, B.R., Zurakowski, D., Macklis, J.D. *J. Neurosci.*, **2000**, 20(19), 7404-7416.
- [73] Macklis, J.D. *J. Neurosci.*, **1993**, 13(9), 3848-3863.
- [74] Macklis, J.D., Yoon, C.H., Snyder, E.Y. *Exp. Neurol.*, **1994**, 129, 9.
- [75] Sheen, V.L., Macklis, J.D. *J. Neurosci.*, **1995**, 15(12), 8378-8392.
- [76] Snyder, E.Y., Macklis, J.D. *Clinical Neurosci.*, **1996**, 3, 310-316.
- [77] Snyder, E.Y., Yoon, C., Flax, J.D., Macklis, J.D. *Proc. Natl. Acad. Sci. USA*, **1997**, 94, 11663-11668.
- [78] Wang, Y., Sheen, V.L., Macklis, J.D. *Exp. Neurol.*, **1998**, 154, 389-402.
- [79] Sheen, V.L., Arnold, M.W., Yang, Y., Macklis, J.D. *Exp. Neurol.*, **1999**, 158, 47-62.
- [80] Parent, J. M., Yu, T. W., Leibowitz, R. T., Geschwind, D. H., Sloviter, R. S., Lowenstein, D. H. *J. Neurosci.*, **1997**, 17, 3727 – 3738.
- [81] Madsen, T.M., Treschow, A., Bengzon, J., Bolwig, T.G., Lindvall, O., Tingstrom, A. *Biol Psychiatry*, **2000**, 47, 1043-1049.
- [82] Jin, K., Peel, A.L., Mao, X.O., Xie, L., Cottrell, B.A., Henshall, D.C., Greenberg, D.A. *Proc. Natl. Acad. Sci. USA*, **2004**, 101(1), 343-7.
- [83] Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O. *Proc. Natl. Acad. Sci. USA*, **2003**, 100(23), 13632-7.
- [84] Monje ML, Toda H, Palmer TD. *Science.*, **2003**, 302(5651), 1760-5.
- [85] Bengzon, J., Kokaia, Z., Eskil Elmér, E., Nanobashvili, A., Kokaia, M., Lindvall, O. *Proc. Natl. Acad. Sci. USA*, **1997**, 94(19), 10432-10437.
- [86] Gray, W.P., Sundstrom, L.E. *Brain Res.*, **1998**, 790, 52-59.
- [87] Parent, J.M., Janumpalli, S., McNamara, J.O., Lowenstein, D.H. *Neurosci. Lett.*, **1998**, 247, 9-12.
- [88] Cameron, H.A., Gould, E. *Neurosci.*, **1994**, 61, 203-209.
- [89] Gould, E., Tanapat, P. *Neuroscience*, **1997**, 80(2), 427-36.
- [90] Liu, J., Solway, K., Messing, R.O., Sharp, F.R. *J. Neurosci.*, **1998**, 18, 7768-7778.
- [91] Bernabeu, R., Sharp, F.R. *J. Cereb. Blood Flow Metab.*, **2000**, 20(12), 1669-1680.
- [92] Kee, N.J., Preston, E., Wojtowicz, J.M. *Exp. Brain Res.*, **2001**, 136, 313-320.
- [93] Arvidsson, A., Kokaia, Z., Lindvall, O. *Europ. J. Neurosci.*, **2001**, 14, 10-18.
- [94] Arvidsson, A., Collin, T., Kirik, D., Kokaia, Z., Lindvall, O. *Nat. Med.*, **2002**, 8(9), 963-70.
- [95] Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM. *Ann. Neurol.*, **2002**, 52(6), 802-13.
- [96] Francis, F., Koulakoff, A., Boucher, D., Chafey, P., Schaar, B., Vinet, M.C., Friocourt, G., McDonnell, N., Reiner, O., Kahn, A., McConnell, S.K., Berwald-Netter, Y., Denoulet, P., Chelly, J. *Neuron*, **1999**, 23, 247-256.
- [97] Gleeson, J.G., Lin, P.T., Flanagan, L.A., Walsh, C.A. *Neuron*, **1999**, 23, 257-271.
- [98] Jin, K., Minami, M., Lan, J.Q., Mao, X.O., Bateur, S., Simon, R.P., Greenberg, D.A. *Proc. Natl. Acad. Sci. USA*, **2001**, 98(8), 4710-5.
- [99] Jin, K., Mao, X.O., Sun, Y., Xie, L., Jin, L., Nishi, E., Klagsbrun, M., Greenberg, D.A. *J. Neurosci.*, **2002c**, 22(13), 5365-73.
- [100] Brown, J.P., Couillard-Despres, S., Cooper-Kuhn, C.M., Winkler, J., Aigner, L., Kuhn, H.G. *J. Comp. Neurol.*, **2003**, 467(1), 1-10.
- [101] Fujita, M., Kiyono, T., Hayashi, Y., Ishibashi, M. *J. Biol. Chem.*, **1996**, 271, 4349-4354.

- [102] Hendzel, M.J., Wei, Y., Mancini, M.A., Van Hooser, A., Ranalli, T., Brinkley, B.R., Bazett-Jones, D.P., Allis, C.D. *Chromosoma*, **1997**, *106*(6), 348-60.
- [103] Ino, H., Chiba, T. *Brain Res. Mol. Brain Res.*, **2000**, *78*, 163-174.
- [104] Ueki, T., Tanaka, M., Yamashita, K., Mikawa, S., Qiu, Z., Maragakis, N.J., Hevner, R.F., Miura, N., Sugimura, H., Sato, K. *J. Neurosci.*, **2003**, *23*(37), 11732-40.
- [105] Kirschenbaum, B., Goldman, S.A. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*, 210-214.
- [106] Craig, C.G., Tropepe, V., Morshead, C.M., Reynolds, B.A., Weiss, S., van der Kooy, D. *J. Neurosci.*, **1996**, *16*, 2649-2658.
- [107] Kuhn, H.G., Winkler, J., Kempermann, G., Thal, L.J., Gage, F.H. *J. Neurosci.*, **1997**, *17*, 5820-5829.
- [108] Gage, F.H., Kempermann, G., Palmer, T.D., Peterson, D.A., Ray, J. *J. Neurobiol.*, **1998**, *36*(2), 249-66.
- [109] Adberg, M.A.I., Adberg, N.D., Hedbäcker, H., Oscarsson, J., Eriksson, P.S. *J. Neurosci.*, **2000**, *20*, 2896-2903.
- [110] Wu, J.-P., Kuo, J.-S., Liu, Y.-L., Tzeng, S.-F. *Neurosci. Lett.*, **2000**, *292*, 203-206.
- [111] Yoshimura, S., Takagi, Y., Harada, J., Teramoto, T., Thomas, S.S., Waeber, C., Bakowska, J.C., Breakefield, X.O., Moskowitz, M.A. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*(10):5874-9.
- [112] Gustafsson, E., Lindvall, O., Kokaia, Z. *Stroke*, **2003**, *34*, 2710-2715.
- [113] Faux, C.H., Turnley, A.M., Epa, R., Cappai, R., Bartlett, P.F. *J. Neurosci.*, **2002**, *21*(15), 5587-5596.
- [114] Zhang, R., Zhang, L., Zhang, Z., Wang, Y., Lu, M., Lapointe, M., Chopp, M. *Ann. Neurol.*, **2001**, *50*, 602-611.
- [115] Mason, H.A., Ito, S., Corfas, G. *J. Neurosci.*, **2001**, *21*, 7654-7663.
- [116] Zhu, D.Y., Liu, S.H., Sun, H.S., Lu, Y.M. *J. Neurosci.*, **2003**, *23*(1), 223-229.
- [117] Moreno-López, B., Romero-Grimaldi, C., Noval, J.A., Murillo-Carretero, M., Matarredona, E.R., Estrada, C. *J. Neurosci.*, **2004**, *24*(1), 85-95.
- [118] Yang, X., Zhong, R., Heintz, N. *Development*, **1996**, *122*, 55-66.
- [119] Sakakibara, S., Imai, T., Hamaguchi, K., Okabe, M., Aruga, J., Nakajima, K., Yasutomi, D., Nagata, T., Kurihara, Y., Uesugi, S., Miyata, T., Ogawa, M., Mikoshiba, K., Okano, H. *Dev. Biol.*, **1996**, *176*(2), 230-42.
- [120] Zindy, F., Cunningham, J.J., Sherr, C.J., Jøgal, S., Smeyne, R.J., Roussel, M.F. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 13462-67.
- [121] Lois, C., Garcia-Verdugo, J.-M., Alvarez-Buylla, A. *Science*, **1996**, *271*, 978.
- [122] Quinn, C.C., Gray, G.E., Hockfield, S. *J. Neurobiol.*, **1999**, *41*, 158-164.
- [123] Muramatsu, T. *J. Biochem.*, **2002**, *132*(3), 359-71.
- [124] Cameron, H.A., McEwen, B.S., Gould, E. *J. Neurosci.*, **1995**, *15*, 4687-4692.
- [125] Gould, E., McEwen, B.S., Tanapat, P., Galea, L.A., Fuchs, E. *J. Neurosci.*, **1997**, *17*, 2492-2498.
- [126] Seki, T., Arai, Y. *Neuroreport*, **1995**, *6*, 2479-2482.
- [127] van Praag, H., Kempermann, G., Gage, F.H. *Nat. Neurosci.*, **1999**, *2*, 266-270.
- [128] Kempermann, G., Kuhn, H.G., Gage, F.H. *Nature*, **1997**, *386*, 493-495.
- [129] Gould, E., Beylin, A., Tanapat, P., Reeves, A., Shors, T.J. *Nature Neurosci.*, **1999**, *2*, 260-265.
- [130] Tanapat, P., Hastings, N.B., Reeves, A.J., Gould, E. *J. Neurosci.*, **1999**, *19*, 5792-5801.
- [131] Rasika, S., Nottebohm, F., Alvarez-Buylla, A. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 7854-7858.
- [132] Brezun, J.M., Daszuta, A. *Neurosci.*, **1999**, *89*, 999-1002.
- [133] Tanapat, P., Galea, L.A., Gould, E. *Int. J. Dev. Neurosci.*, **1998**, *16*, 235-239.
- [134] Cameron, H.A., Tanapat, P., Gould, E. *Neurosci.*, **1998**, *82*, 349-354.
- [135] Stein-Behrens, B.A., Lin, W.J., Salpolsky, R.M. *J. Neurochem.*, **1994**, *63*, 596-602.
- [136] Chen, G., Rajkowska, G., Du, F., Seraji-Bozorgzad, N., Manji, H.K. *J. Neurochem.*, **2000**, *75*(4), 1729-1734.
- [137] Malberg, J.E., Eisch, A.J., Nestler, E.J., Duman, R.S. *J. Neurosci.*, **2000**, *20*, 9104-9110.
- [138] Wechsler-Reya, R.J., Scott, M.P. *Neuron*, **1999**, *22*(1), 103-114.
- [139] Kohtz, J.D., Baker, D.P., Corte, G., Fishell, G. *Development*, **1998**, *125*(24), 5079-89.
- [140] Sussel, L., Marin, O., Kimura, S., Rubenstein, J.L. *Development*, **1999**, *126*(15), 3359-70.
- [141] Patten, I., Placzek, M. *Cell Mol. Life Sci.*, **2000**, *57*, 1695-1708.
- [142] Deshpande, G., Swanhart, L., Chiang, P., Schedl, P. *Cell*, **2001**, *106*, 759-769.
- [143] Nery, S., Wichterle, H., Fischell, G. *Development*, **2001**, *128*, 527-540.
- [144] Tekki-Kassar, N., Woodruff, R., Hall, A.C., Gaffield, W., Kimura, S., Stiles, C.D., Rowitch, D.H., Richardson, W.D. *Development*, **2001**, *128*(13), 2545-54.
- [145] Testaz, S., Jarov, A., Williams, K.P., Ling, L.E., Koteliensky, V.E., Fournier-Thibault, C., Duband, J.L. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 12521-12526.
- [146] Orentas, D.M., Miller, R.H. *Dev. Biol.*, **1996**, *177*(1), 43-53.
- [147] Dahmane, N., Ruiz-i-Altaba, A. *Development*, **1999**, *126*(14), 3089-100.
- [148] Rowitch, D.H., S-Jacques, B., Lee, S.M., Flax, J.D., Snyder, E.Y., McMahon, A.P. *J. Neurosci.*, **1999**, *19*(20), 8954-65.
- [149] Wallace, V.A. *Curr. Biol.*, **1999**, *9*(8), 445-8.
- [150] Zhu, G., Mehler, M.F., Zhao, J., Yu Yung, S., Kessler, J.A. *Dev. Biol.*, **1999**, *215*, 118-129.
- [151] Davies, J.E., Miller, R.H. *Dev. Biol.*, **2001**, *233*, 513-525.
- [152] Gulacsi, A., Lillien, L. *J. Neurosci.*, **2003**, *23*(30), 9862-9872.
- [153] Jarman, A.P. *Curr. Biol.*, **2000**, *10*, R857-859.
- [154] Hammerschmidt, M., Brook, A., McMahon, A.P. *Trends Genet.*, **1997**, *13*, 14-21.
- [155] Marti, E., Takada, R., Bumcrot, D.A., Sasaki, H., McMahon, A.P. *Development*, **1995**, *121*(8), 2537-47.
- [156] Roelink, H., Porter, J.A., Chiang, C., Tanabe, Y., Chang, D.T., Beachy, P.A., Jessell, T.M. *Cell*, **1995**, *81*(3), 445-55.
- [157] Chiang, C., Litingtung, Y., Lee, E., Young, K.E., Corden, J.L., Westphal, H., Beachy, P.A. *Nature*, **1996**, *383*, 407-413.
- [158] Ericson, J., Morton, S., Kawakami, A., Roelink, H., Jessell, T.M. *Cell*, **1996**, *87*(4), 661-73.
- [159] Jensen, A.M., Wallace, V.A. *Development*, **1997**, *124*, 363-371.
- [160] Levine, E.M., Roelink, H., Turner, J., Reh, T.A. *J. Neurosci.*, **1997**, *17*(16), 6277-88.
- [161] Briscoe, J., Ericson, J. *Semin. Cell Dev. Biol.*, **1999**, *10*, 353-362.
- [162] Jessell, T.M. *Nat. Rev. Genet.*, **2000**, *1*, 20-29.
- [163] Hynes, M., Ye, W., Wang, K., Stone, D., Murone, M., Sauvage, F., Rosenthal, A. *Nat. Neurosci.*, **2000**, *3*, 41-46.
- [164] Briscoe, J., Chen, Y., Jessell, T.M., Struhl, G. *Mol. Cell.*, **2001**, *7*, 1297-1291.
- [165] Hirsinger, E., Duprez, D., Jouve, C., Malapert, P., Cooke, J., Pourquie, O. *Development*, **1997**, *124*(22), 4605-14.
- [166] Roessler, E., Belloni, E., Gaudenz, K., Jay, P., Berta, P., Scherer, S.W., Tsui, L.C., Muenke, M. *Nat. Genet.*, **1996**, *14*, 357-360.
- [167] Wechsler-Reya, R., Scott, M.P. *Annu. Rev. Neurosci.*, **2001**, *24*, 385-428.
- [168] Tabin, C.J., McMahon, A.P. *Trends Cell Biol.*, **1997**, *7*, 442-446.
- [169] Van del Heuvel, M., Ingham, P.W. *Nature*, **1996**, *382*, 547-551.
- [170] Wodarz A., Nusse R. *Annu. Rev. Cell Dev. Biol.*, **1998**, *14*, 59-88.
- [171] Ming, J.E., Roessler, E., Muenke, M. *Mol. Med. Today.*, **1998**, *4*(8), 343-9.
- [172] Xie, J., Aszterbaum, M., Zhang, X., Bonifas, J.M., Zachary, C., Epstein, E., McCormick, F. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*(16), 9255-9.
- [173] Hahn, H., Wójnowski, L., Specht, K., Kappler, R., Calzada-Wack, J., Potter, D., Zimmer, A., Müller, U., Samson, E., Quintanilla-Martinez, L., Zimmer, A. *J. Biol. Chem.*, **2000**, *275*(37), 28341-4.
- [174] Nicot, A., Lelievre, V., Tam, J., Waschek, J.A., DiCicco-Bloom, E. *J. Neurosci.*, **2002**, *22*(21), 9244-54.
- [175] Nybakken, K., Perrimon, N. *Curr. Opin. Genet. Dev.*, **2002**, *12*, 503-511.
- [176] Kenney, A.M., Cole, M.D., Rowitch, D.H. *Development*, **2003**, *130*(1), 15-28.
- [177] Oliver, T.G., Grasfeder, L.L., Carroll, A.L., Kaiser, C., Gillingham, C.L., Lin, S.M., Wickramasinghe, R., Scott, M.P., Wechsler-Reya, R.J. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*(12), 7331-6.
- [178] Alcedo, J., Zou, Y., Noll, M. *Mol. Cell.*, **2000**, *6*, 457-465.
- [179] Deneff, N., Neubuser, D., Perez, L., Cohen, S.M. *Cell*, **2000**, *102*, 521-531.
- [180] Ingham, P.W., Nystedt, S., Nakano, Y., Brown, W., Stark, D., van den Heuvel, M., Taylor, A.M. *Curr. Biol.*, **2000**, *10*, 1315-1318.
- [181] Gallet, A., Angelats, C., Kerridge, S., Therond, P.P. *Development*, **2000**, *127*, 5509-5522.

- [182] Traiffort, E., Charytoniuk, D., Watroba, L., Faure, H., Sales, N., Ruat, M. *Eur. J. Neurosci.*, **1999**, *11*(9), 3199-214.
- [183] Lai, K., Kaspar, B.K., Gage, F.H., Schaffer, D.V. *Nat. Neurosci.*, **2003**, *6*(1), 21-27.
- [184] Amaral, D.G., Kurz, J. *J. Comp. Neurol.*, **1985**, *240*, 37-59.
- [185] Tsuboi, K., Shults, C.W. *Exp. Neurol.*, **2002**, *173*(1), 95-104.
- [186] Pepinsky, R.B., Shapiro, R.I., Wang, S., Chakraborty, A., Gill, A., Lepage, D.J., Wen, D., Rayhorn, P., Horan, G.S., Taylor, F.R., Garber, E.A., Galdes, A., Engber, T.M. *J. Pharm. Sci.*, **2002**, *91*(2), 371-87.
- [187] Bambakidis, N.C., Wang, R.Z., Franic, L., Miller, R.H. *J. Neurosurg.*, **2003**, *99*(suppl.), 70-75.
- [188] Frank-Kamenetsky, M., Zhang, X.M., Bottega, S., Guicherit, O., Wichterle, H., Dudek, H., Bumcrot, D., Wang, F.Y., Jones, S., Shulok, J., Rubin, L.L., Porter, J.A. *J. Biol.*, **2002**, *1*(2), 10.
- [189] King, R.W. *J. Biol.*, **2002**, *1*, 8.
- [190] Taipale, J., Chen, J.K., Cooper, M.K., Wang, B., Mann, R.K., Milenkovic, L., Scott, M.P., Beachy, P.A. *Nature*, **2000**, *406*(6799), 1005-9.
- [191] Williams, J.A., Guicherit, O.M., Zaharian, B.I., Xu, Y., Chai, L., Wichterle, H., Kon, C., Gatchalian, C., Porter, J.A., Rubin, L.L., Wang, F.Y. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*(8), 4616-21.
- [192] Chen, J.K., Taipale, J., Young, K.E., Maiti, T., Beachy, P.A. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*(22), 14071-6.
- [193] Stecca, B., Altaba, A.R. *J. Biol.*, **2002**, *1*, 9.
- [194] Dohlman, H.G., Thorner, J., Caron, M.G., Lefkowitz, R.J. *Annu. Rev. Biochem.*, **1991**, *60*, 653-88.
- [195] Weitzman, J.B. *J. Biol.*, **2002**, *1*, 7.
- [196] Urist, M.R. *Science*, **1965**, *150*(698), 893-9.
- [197] Urist, M.R., Strates, B.S. *J. Dent. Res.*, **1971**, *50*(6), 1392-406.
- [198] Baker, J.C., Beddington, R.S., Harland, R.M. *Genes Dev.*, **1999**, *13*(23), 3149-59.
- [199] Finley, M.F., Devata, S., Huettner, J.E. *J. Neurobiol.*, **1999**, *40*(3), 271-87.
- [200] Sasai, Y. *Curr. Opin. Neurobiol.*, **2001**, *11*(1), 22-6.
- [201] Wessely, O., Agius, E., Oelgeschlager, M., Pera, E.M., De Robertis, E.M. *Dev. Biol.*, **2001**, *234*(1), 161-73.
- [202] Mehler, M.F., Mabie, P.C., Zhu, G., Gokhan, S., Kessler, J.A. *Dev. Neurosci.*, **2000**, *22*(1-2), 74-85.
- [203] Anderson, D., Groves, A., Lo, L., Ma, Q., Rao, M., Shah, N.M., Sommer, L. *Cold Spring Harbour Symposium Quant. Biol.*, **1997**, *62*, 493-504.
- [204] Lo, L., Sommer, L., Anderson, D.J. *Curr. Biol.*, **1997**, *7*(6), 440-450.
- [205] Gross, R., Mehler, M., Mabie, P., Zang, Z., Santschi, L., Kessler, J. *Neuron*, **1996**, *17*, 595-606.
- [206] Mabie, P.C., Mehler, M.F., Kessler, J.A. *J. Neurosci.*, **1999**, *19*(16), 7077-88.
- [207] Roberts, D.J., Johnson, R.L., Burke, A.C., Nelson, C.E., Morgan, B.A., Tabin, C. *Development*, **1995**, *121*(10), 3163-74.
- [208] Lillien, L., Raphael, H. *Development*, **2000**, *127*, 4993-5005.
- [209] Kalyani, A.J., Piper, D., Mujtaba, T., Lucero, M.T., Rao, M.S. *J. Neurosci.*, **1998**, *18*, 7856-7868.
- [210] Li, W., Cogswell, C.A., LoTurco, J.J. *J. Neurosci.*, **1998**, *18*, 8853-8862.
- [211] Yung, S.Y., Gokhan, S., Jurcsak, J., Molero, A.E., Abrajano, J.J., Mehler, M.F. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*(25), 16273-8.
- [212] Yamato, K., Hashimoto, S., Okahashi, N., Ishisaki, A., Nonaka, K., Koseki, T., Kizaki, M., Ikeda, Y., Nishihara, T. *Exp. Cell Res.*, **2000**, *257*(1), 198-205.
- [213] Sampath, T.K., Coughlin, J.E., Whetstone, R.M., Banach, D., Corbett, C., Ridge, R.J., Ozkaynak, E., Oppermann, H., Rueger, D.C. *J. Biol. Chem.*, **1990**, *265*(22), 13198-205.
- [214] Aono, A., Hazama, M., Notoya, K., Taketomi, S., Yamasaki, H., Tsukuda, R., Sasaki, S., Fujisawa, Y. *Biochem. Biophys. Res. Commun.*, **1995**, *210*(3), 670-7.
- [215] Soderstrom, S., Bengtsson, H., Ebendal, T. *Cell Tissue Res.*, **1996**, *286*(2), 269-79.
- [216] Mehler, M.F., Mabie, P.C., Zhang, D., Kessler, J.A. *Trends Neurosci.*, **1997**, *20*, 309-317.
- [217] Zhang, D., Mehler, M.F., Song, Q., Kessler, J.A. *J. Neurosci.*, **1998**, *18*, 3314-3326.
- [218] Zwijssen, A., Verschueren, K., Huylebroeck, D. *FEBS Lett.*, **2003**, *546*(1), 133-9.
- [219] Hogan, B.L. *Genes Dev.*, **1996**, *10*(13), 1580-94.
- [220] Massague, J. *Cell*, **1996**, *85*, 947-950.
- [221] ten Dijke, P., Miyazono, K., Heldin, C.H. *Curr. Opin. Cell Biol.*, **1996**, *8*(2), 139-45.
- [222] Rosenzweig, B.L., Imamura, T., Okadome, T., Cox, G.N., Yamashita, H., ten Dijke, P., Heldin, C.H., Miyazono, K. *Proc. Natl. Acad. Sci. USA*, **1995**, *92*(17), 7632-6.
- [223] Liu, F., Ventura, F., Doody, J., Massague, J. *Mol. Cell Biol.*, **1995**, *15*(7), 3479-86.
- [224] Piccolo, S., Sasai, Y., Lu, B., De Robertis, E.M. *Cell*, **1996**, *86*(4), 589-98.
- [225] Zimmerman, L.B., De Jesus-Escobar, J.M., Harland, R.M. *Cell*, **1996**, *86*(4), 599-606.
- [226] Holley, S.A., Neul, J.L., Attisano, L., Wrana, J.L., Sasai, Y., O'Connor, M.B., De Robertis, E.M., Ferguson, E.L. *Cell*, **1996**, *86*(4), 607-17.
- [227] Balemans, W., Van Hul, W. *Dev. Biol.*, **2002**, *250*(2), 231-50.
- [228] Reynisdottir, I., Polyak, K., Iavarone, A., Massague, J. *Genes Dev.*, **1995**, *9*(15), 1831-45.
- [229] Nohe, A., Keating, E., Knaus, P., Petersen, N.O. *Cell Signal.*, **2004**, *16*(3), 291-9.
- [230] Tomizawa, K., Matsui, H., Kondo, E., Miyamoto, K., Tokuda, M., Itano, T., Nagahata, S., Akagi, T., Hatase, O. *Brain Res. Mol. Brain Res.*, **1995**, *28*(1), 122-8.
- [231] Martinez, G., Carnazza, M.L., Di Giacomo, C., Sorrenti, V., Vanella, A. *Brain Res.*, **2001**, *894*(1), 1-11.
- [232] Lewen, A., Soderstrom, S., Hillered, L., Ebendal, T. *Neuroreport*, **1997**, *8*(2), 475-9.
- [233] Charytoniuk, D.A., Traiffort, E., Pinard, E., Issertial, O., Seylaz, J., Ruat, M. *Neuroscience*, **2000**, *100*(1), 33-43.
- [234] Wang, Y., Chang, C.F., Morales, M., Chou, J., Chen, H.L., Chiang, Y.H., Lin, S.Z., Cadet, J.L., Deng, X., Wang, J.Y., Chen, S.Y., Kaplan, P.L., Hoffer, B.J. *Stroke*, **2001**, *32*(9), 2170-8.
- [235] Alder, J., Lee, K.J., Jessell, T.M., Hatten, M.E. *Nat. Neurosci.*, **1999**, *2*(6), 535-40.
- [236] Perides, G., Jensen, F.E., Edgecomb, P., Rueger, D.C., Charness, M.E. *Neurosci. Lett.*, **1995**, *187*(1), 21-4.
- [237] Lin, S.Z., Hoffer, B.J., Kaplan, P., Wang, Y. *Stroke*, **1999**, *30*(1), 126-33.
- [238] Kawamata, T., Ren, J., Chan, T.C., Charette, M., Finklestein, S.P. *Neuroreport*, **1998**, *9*(7), 1441-5.
- [239] Ren, J., Kaplan, P.L., Charette, M.F., Speller, H., Finklestein, S.P. *Neuropharmacology*, **2000**, *39*(5), 860-5.
- [240] Chang, C.F., Lin, S.Z., Chiang, Y.H., Morales, M., Chou, J., Lein, P., Chen, H.L., Hoffer, B.J., Wang, Y. *Stroke*, **2003**, *34*(2), 558-64.
- [241] Seri, B., Garcia-Verdugo, J.M., McEwen, B.S., Alvarez-Buylla, A. *J. Neurosci.*, **2001**, *21*, 7153-7160.
- [242] Gotz, M. *Neuroscientist*, **2003**, *9*(5), 379-97.
- [243] Ingber, D., Fujita, T., Kishimoto, S., Sudo, K., Kanamaru, T., Brem, H., Folkman, J. *Nature*, **1990**, *348*(6301), 555-7.
- [244] Leid, M., Kastner, P., Chambon, P. *Trends Biochem. Sci.*, **1992**, *17*(10), 427-33.
- [245] Kusaka, M., Sudo, K., Matsutani, E., Kozai, Y., Marui, S., Fujita, T., Ingber, D., Folkman, J. *Br. J. Cancer*, **1994**, *69*(2), 212-6.
- [246] Abe, J., Zhou, W., Takuwa, N., Taguchi, J., Kurokawa, K., Kumada, M., Takuwa, Y. *Cancer Res.*, **1994**, *54*(13), 3407-12.
- [247] Nagy, L., Thomazy, V.A., Shipley, G.L., Fesus, L., Lamph, W., Heyman, R.A., Chandraratna, R.A., Davies, P.J. *Mol. Cell Biol.*, **1995**, *15*(7), 3540-51.
- [248] Glozak, M.A., Rogers, M.B. *Exp. Cell Res.*, **1998**, *242*(1), 165-73.
- [249] Mori, S., Yoshikawa, H., Hashimoto, J., Ueda, T., Funai, H., Kato, M., Takaoka, K. *Bone*, **1998**, *22*(2), 99-105.
- [250] Edwards, C.J. *Ann. Acad. Med. Singapore*, **2002**, *31*(2), 245-7.
- [251] Seeherman, H., Wozney, J., Li, R. *Spine*, **2002**, *27*(16 Suppl. 1), S16-23.
- [252] LaRosa, J.C., He, J., Vupputuri, S. *JAMA*, **1999**, *282*(24), 2340-6.
- [253] Mundy, G., Garrett, R., Harris, S., Chan, J., Chen, D., Rossini, G., Boyce, B., Zhao, M., Gutierrez, G. *Science*, **1999**, *286*(5446), 1946-9.
- [254] Edwards, C.J., Spector, T.D. *Arthritis Res.*, **2002**, *4*(3), 151-3.
- [255] Madsen, T.M., Newton, S.S., Eaton, M.E., Russell, D.S., Duman, R.S. *Biol. Psychiat.*, **2003**, *54*(10), 1006-1014.
- [256] Chenn, A., Walsh, C.A. *Science*, **2002**, *297*, 365-369.
- [257] Vinson, C.R., Conover, S., Adler, P.N. *Nature*, **1989**, *338*, 263-64.
- [258] Patapoutian, A., Reichardt, L.F. *Curr. Opin. Neurobiol.*, **2000**, *10*, 392-399.
- [259] van de Wetering, M., Sancho, E., Verweij, C., de Lau, W., Oving, I., Hurlstone, A. *Cell*, **2002**, *111*, 241-250.

- [260] Nishimura, W., Yao, I., Iida, J., Tanaka, N., Hata, Y. *J. Neurosci.*, **2002**, *22*, 757-765.
- [261] Leyns, L., Bouwmeester, T., Kim, S.H., Piccolo, S., De Robertis, E. *Cell.*, **1997**, *88*, 747-56.
- [262] Lin, K., Wang, S., Julius, M.A., Kitajewski, J., Moos, M.J., Luyten, F.P. *Proc. Natl. Acad. Sci. USA*, **1997**, *94*, 11196-200.
- [263] Hall, A.C., Lucas, F.R., Salinas, P.C. *Cell*, **2000**, *100*, 525-535.
- [264] Krylova, O., Herreros, J., Cleverley, K.E., Ehler, E., Henriquez, J.P., Hughes, S.M., Salinas, P.C. *Neuron*, **2002**, *35*, 1043-1056.
- [265] Murase, S., Mosser, E., Schuman, E.S., *Neuron*, **2002**, *35*, 91-105.
- [266] Galceran, J., Miyashita-Lin, E.M., Devaney, E., Rubenstein, J.L., Grosschedl, R. *Development*, **2000**, *127*, 469-482.
- [267] Lee, J.C., Mayer-Proschel, M., Rao, M.S. *Glia*, **2000**, *30*(2), 105-21.
- [268] Lijam, N., Paylor, R., McDonald, M.P., Crawley, J.N., Deng, C.X., Herrup, K. *Cell*, **1997**, *90*, 895-905.
- [269] Spittaels, K., Van den Haute, C., Van Dorpe, J., Terwel, D., Vandezande, K., Lasrado, R., Bruynseels, K., Irizarry, M., Verhoye, M., Van Lint, J., Vandenheede, J.R., Ashton, D., Mercken, M., Loos, R., Hyman, B., Van der Linden, A., Geerts, H., Van Leuven, F. *Neuroscience*, **2002**, *113*(4), 797-808.
- [270] Brault, V., Moore, R., Kutsch, S., Ishibashi, M., Rowitch, D.H., McMahon, A.P. *Development*, **2001**, *128*, 1253-1264.
- [271] De Ferrari, D.V., Inestrosa, N.C. *Brain Res. Brain Res. Rev.*, **2000**, *33*, 1-12.
- [272] Li, J.L., Jing, Z.Z., Yi, H. *Hunan Yi Ke Da Xue Xue Bao.*, **2003**, *28*(4), 330-4.
- [273] Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., *Science*, **2003**, *301*, 805-809.
- [274] Jope, R.S., Bijur, G.N. *Mol. Psychiatry.*, **2002**, *7*, S35-S45.
- [275] Hall, A.C., Brennan, A., Goold, R.G., Cleverley, K., Lucas, F.R., Gordon-Weeks, P.R., Salinas, P.C. *Mol. Cell. Neurosci.*, **2002**, *20*, 257-270.
- [276] Mai, L., Jope, R.S., Li, X. *J. Neurochem.*, **2002**, *82*, 75-83.
- [277] Wagman, A.S., Johnson, K.W., Bussiere, D.E. *Curr. Pharm. Des.*, **2004**, *10*(10), 1105-37.
- [278] Fishman, P., Madi, L., Bar-Yehuda, S., Barer, F., Del Valle, L., Khalili, K. *Oncogene*, **2002**, *21*(25), 4060-4.
- [279] Fishman, P., Bar-Yehuda, S. *Curr. Top. Med. Chem.*, **2003**, *3*(4), 463-9.
- [280] Chen, G., Rajkowska, G., Du, F., Seraji-Bozorgzad, N., Manji, H.K. *J. Neurochem.*, **2000**, *75*(4), 1729-1734.
- [281] Catapano, L.A., Arnold, M.W., Perez, F.A., Macklis, J.D. *J. Neurosci.*, **2001**, *21*, 8863-8872.
- [282] Nottebohm, F. *Brain Res. Bull.*, **2002**, *57*(6), 737-49.
- [283] Arsenijevic, Y., Weiss, S., Schneider, B., Aebischer, P. *J. Neurosci.*, **2001**, *21*(18), 7194-202.
- [284] Kuhn, H.G., Palmer, T.D., Fuchs, E. *Eur. Arch. Psychiatry Clin. Neurosci.*, **2001**, *251*, 152-158.
- [285] Wang, S., Scott, B.W., Wojtowicz, J.M. *J. Neurobiol.*, **2000**, *42*, 248-257.
- [286] Snyder, J.S., Kee, N., Wojtowicz, J.M. *J. Neurophysiol.*, **2001**, *85*, 2423-2431.
- [287] Raballo, R., Rhee, J., Lyn-Cook, R., Leckman, J.F., Schwartz, M.L., Vaccarino, F.M. *J. Neurosci.*, **2000**, *20*(13), 5012-23.
- [288] Vaccarino, F.M., Schwartz, M.L., Raballo, R., Nilsen, J., Rhee, J., Zhou, M., Doetschman, T., Coffin, J.D., Wyland, J.J., Hung, Y.T. *Nat. Neurosci.*, **1999**, *2*(9), 848.
- [289] Gritti, A., Frolichsthal-Schoeller, P., Galli, R., Parati, E.A., Cova, L., Pagano, S.F., Bjornson, C.R., Vescovi, A.L. *J. Neurosci.*, **1999**, *19*(9), 3287-97.
- [290] Tropepe, V., Sibilina, M., Ciruna, B.G., Rossant, J., Wagner, E.F., van der Kooy, D. *Dev. Biol.*, **1999**, *208*(1), 166-88.
- [291] Gaiano, N., Kohtz, J.D., Turnbull, D.H., Fishell, G. *Nat. Neurosci.*, **1999**, *2*, 812-819.
- [292] Machold, R., Hayashi, S., Rutlin, M., Muzumdar, M.D., Nery, S., Corbin, J.G., Gritti-Linde, A., Dellovade, T., Porter, J.A., Rubin, L.L., Dudek, H., McMahon, A.P., Fishell, G. *Neuron*, **2003**, *39*(6):937-50.
- [293] Abate-Shen, C., Shen, M.M. *Genes Dev.*, **2000**, *14*, 2410-2434.
- [294] Zumkeller, W., Westphal, M. *Mol. Pathol.*, **2001**, *54*, 227-229.
- [295] Kwan, H., Pecsenka, V., Tsukamoto, A., Parslow, T.G., Guzman, R., Lin, T.P., Muller, W.J. *Mol. Cell Biol.*, **1992**, *12*(1), 147-54.
- [296] Shackelford, G.M., MacArthur, C.A., Kwan, H.C., Varmus, H.E. *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 740-744.
- [297] Lee, C.S., Buttiitta, L., Fan, C.M. *Proc. Natl. Acad. Sci. USA*, **2001**, *98*, 11347-11352.
- [298] Detmer, K., Walker, A.N., Jenkins, T.M., Steele, T.A., Dannawi, H. *Blood Cells Mol. Dis.*, **2000**, *26*(4), 360-72.