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Review

Genetic models to study adult neurogenesis✪

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In the central nervous system (CNS) generation of new neurons continues throughout adulthood, when it is limited to the olfactory bulb and hippocampus. The knowledge regarding the function of newly-generated neurons remains limited and is vigorously investigated using diverse approaches. Among these are genetically modified mice, most of them of knock-out type (KO). Results from 23 diverse KO mouse models demonstrate the importance of particular proteins (growth factors, nitric oxide synthases, receptors, cyclins/cyclin-associated proteins, transcription factors, etc.) in adult neurogenesis (ANGE) as well as separate it from developmental neurogenesis. These results bring us closer to revealing the function of newly generated neurons in adult brains.

Keywords: knock-out mice, brain, hippocampus, dentate gyrus, olfactory bulb, BrdU.

The term adult neurogenesis (ANGE) refers to generation of new neurons (due to the proliferation of precursor cells and their differentiation) in the brains of adult animals. Mammalian ANGE was initially observed decades ago (Messier *et al.*, 1958; Messier & Leblond, 1960; Smart, 1961; Altman & Das, 1965; 1966) but has only recently gained recognition. ANGE is limited to two populations of dividing cells: (i) those in the subventricular zone (SVZ) with its projection through the rostral migratory stream to the olfactory bulb (OB), and (ii) those in the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. Those cells mature into OB granule cells and DG granule cells, respectively. ANGE is regulated by a multitude of environmental and physiological stimuli (see Table 1 in Eisch & Nestler, 2002). Lately, several genetic manipulations have also proved effective in altering ANGE (see below and Tables 1 and 2).

The first indication that genes influence ANGE came from studies on neurogenesis in mice of different genetic background. Various mouse strains (C57BL/6, BALB/c, CD1(ICR), 129Sv/J A/J, C3H/HeJ and DBA/2J) show distinct rates of proliferation, survival, and differentiation of newborn cells in DG (Kempermann & Gage, 2002; Kempermann *et al.*, 1997a). Also, environmental stimulation differentially influences cell proliferation and survival in C57BL/6 and 129/SvJ mouse strains (Kempermann *et al.*, 1997b; 1998a; 1998b). These results pointed out that diverse aspects of ANGE in the hippocampus are differentially influenced by the genetic background.

There are several genetically modified mouse strains with introduced expression or overexpression of transgenes affecting ANGE (German & Eisch, 2004). In this review, however, we have concentrated on knock-out mice.

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Abbreviations: ANGE, adult neurogenesis; BDNF, brain-derived neurotrophic factor; CB, cannabinoid; CB1, cannabinoid receptor; CNS, central nervous system; Cox, cyclooxygenase; DG, dentate gyrus; eNOS, endothelial NOS; FB, forebrain-specific; Fgf2, basic fibroblast growth factor, bFGF; GFAP, glial fibrillary acidic protein; GIP, gastric inhibitory polypeptide; GIPR, gastric inhibitory polypeptide receptor; GR, glucocorticoid receptor; HMG, high-mobility-group (proteins); 5-HT, serotonin; 5-HT1AR, serotonin receptor 1A; IGF1, insulin-like growth factor 1; iNOS, inducible NOS; KO, knock-out (mouse); LTP, long-term potentiation; mCD24, mouse Cluster and Differentiation 24, glycosylphosphatidylinositol-anchored molecule; MPS III B, mucopolysaccharidosis III type B; MR, mineralocorticoid receptor; NK1R, neurokinin-1 receptor; nNOS, neuronal NOS; NOS, nitric oxide synthase; OB, olfactory bulb; PS1, presenilin-1; SGZ, subgranular zone; Sox, SRY-related HMG box gene family; SP, substance P; SVZ, subventricular zone; VR1, vanilloid receptor.

Table 1. Effects of KO mutations on ANGE (adult neurogenesis)

Gene/Protein	Name/Function	Effects on ANGE	Site	Reference
Cyclin D2	cell cycle regulatory protein	complete reduction	DG, OB	Kowalczyk *et al.*, 2004
tlx(*tailless*)/Tlx	transcription factor	complete reduction	DG, SVZ	Shi *et al.*, 2004
nNOS	neuronal NO synthase	enhancement	SVZ, OB, DG	Packer *et al.*, 2003
eNOS	endothelial NO synthase	reduction	SVZ	Reif *et al.*, 2004
BDNF	growth factor	reduction[b]	DG	Lee *et al.*, 2002
Fgf2	growth factor	reduction	SVZ	Zheng *et al.*, 2004
IGF1	insulin-like growth factor	enhancement[a]	DG	Cheng *et al.*, 2001
CB1R	cannabinoid receptor	reduction	DG, SVZ	Jin *et al.*, 2004
VR1	vanilloid receptor	enhancement	DG, SVZ	Jin *et al.*, 2004
GIPR	GIP receptor	reduction	DG	Nyberg *et al.*, 2005
MR	mineralocorticoid receptor	reduction	DG	Gass *et al.*, 2000
NK1R	neurokinin-1 receptor	enhancement[a]	DG	Morcuende *et al.*, 2003
mCD24	membrane-associated molecule	enhancement[a,c]	DG, SVZ	Belvindrah *et al.*, 2002
Sox2	transcription factor	reduction[b]	DG, SVZ	Ferri *et al.*, 2004
p27Kip1	cyclin-dependent kinase inhibitor	enhancement[c]	SVZ	Doetsch *et al.*, 2002
Naglu	α-*N*-acetylglucosaminidase	reduction	DG, SVZ	Li *et al.*, 2002
Cystatin C	cysteine protease inhibitor	reduction	DG	Pirttila *et al.*, 2004
Bax	proapoptotic Bcl-2	enhancement	DG	Sun *et al.*, 2004

[a]The effect is masked by increased apoptosis; [b]mice used were not null mutants (see text for details); [c]increased was the number of specific cell type (see text for details).

GENETIC MANIPULATIONS RESULTING IN A LOSS OF ADULT NEUROGENESIS: CYCLIN D2 AND TRANSCRIPTION FACTOR Tlx

Gene targeting is a relatively new approach to study mechanisms underlying ANGE. In fact, few have imagined finding a KO mouse with perfect developmental neurogenesis and a selective deficit of the adult one. Mice from several KO strains with affected developmental neurogenesis die before adulthood (e.g., Di Cunto *et al.*, 2000). Nevertheless, in two cases investigators were able to show complete ablation of ANGE in grown-up KO mice. These cases include cyclin D2 KO mice and orphan nuclear receptor/*tailless* (Tlx) KO mice.

Cyclins D are cell cycle regulatory proteins that control specific cyclin-dependent kinases. Three cyclins D have been described: D1, D2, and D3. In most cells, more than one cyclin D is expressed. However, in those instances where only one cyclin D is expressed, its mutation produces significant phenotypic abnormalities (Sicinski *et al.*, 1995; Ma *et al.*, 1998; Sicinska *et al.*, 2003). Among them, mice lacking cyclin D2 display tissue-specific abnormalities in the ovaries and testes (Robker & Richards, 1998; Sicinski *et al.*, 1996), in B cells (Solvason *et al.*, 2000), and in the cerebellum (Huard *et al.*, 1999).

Using these mice (cyclin D2 KO), we discovered a lack of newly born neurons in adult DG and OB. In contrast, ANGE appears normal in cyclin D1 KO mice as well as in the olfactory epithelium of D2 KO mice. Furthermore, cyclin D2 is the only D-type cyclin expressed in dividing cells derived from neuronal precursors present in the adult hippocampus. In contrast, all three cyclin D mRNAs are present in cultures derived from 5-day-old hippocampi, when developmental neurogenesis in DG takes place. The lack of ANGE resulted in changes in adult brain architecture, especially smaller OB, hippocampus, cerebellum, and sensory cortex (Kowalczyk *et al.*, 2004).

Tlx is a forebrain-restricted transcription factor. It was initially identified as an orphan nuclear receptor expressed in vertebrate forebrains (Yu *et al.*, 1994). Expression of Tlx is high at embryonic day 13.5 and in the adult brain (Monaghan *et al.*, 1995), where it is expressed sparsely throughout the cortex and highly in SVZ and DG in adult neural stem cells or progenitor cells. These latter observations were possible thanks to the use of β-galactosidase reporter, which was knocked into the *Tlx* locus (Shi *et al.*, 2004). The *Tlx* gene regulates the timing of neurogenesis in the cortex indicating that Tlx is an essential intrinsic regulator in the decision to proliferate or differentiate in the developing forebrain (Roy *et al.*, 2004).

The brains of Tlx-null mice have been reported to have no obvious defects during embryogenesis and appear grossly normal at birth; however, mature mice suffer from retinopathies, severe limbic defects, reduced cerebral hemispheres and DGs, greatly expanded lateral ventricles and reduced olfactory bulbs. Behaviorally, Tlx mutants show aggressiveness, reduced copulation and progressively violent behavior (Monaghan *et al.*, 1997; Yu *et al.*, 2000).

Nestin is a common marker of proliferating CNS progenitors (Lendahl *et al.*, 1990; Reynolds *et al.*, 1992). Tlx mutant mice show loss of cell proliferation and reduced labeling of nestin in neurogenic

Table 2. Some genetic model organisms displaying effects on ANGE in specific experimental situations.

Gene/ Protein	Name/Function	Experimental situation	Effects on neurogenesis	References
iNOS	inducible NO synthase	ischemia	decrease in ipsilateral DG	Zhu *et al.*, 2003
NK1R	neurokinin-1 receptor	chronic antidepressants	lack of increase in DG	Morcuende *et al.*, 2003
PS1	presenilin-1	enriched environment	lower level in DG	Feng *et al.*, 2004
NMDAR ε 1	receptor subunit	running wheel	lack of increase in DG	Kitamura *et al.*, 2003
Cox-2	cyclooxygenase enzyme	ischemia	lower level in DG	Sasaki *et al.*, 2003
5-HT1AR	serotonin receptor	fluoxetine (antidepressant)	lack of increase in DG	Santarelli *et al.*, 2003
Cystatin C	cysteine protease inhibitor	status epilepticus	decreased migration in DG	Pirttila *et al.*, 2004

areas in the adult brain. The importance of Tlx was further supported by the observation that wild-type Tlx-expressing cells isolated from adult brains can proliferate, self-renew and differentiate into all neural cell types, while Tlx-null cells isolated from adult mutant brains fail to proliferate and self-renew. This latter phenotype is rescued by reintroducing Tlx into Tlx-null cells (Shi *et al.*, 2004).

Tlx KO and D2 KO adult mutant brains have a number of morphological similarities. Both show severely reduced structures including DG, OB as well as greatly expanded lateral ventricles and shrinkage of the medial-posterior cortex. Notably, this phenotype was also observed in other KO mice with altered ANGE intensity (compare Fig. 6 in Ferri *et al.*, 2004, and Fig. 3 in Kowalczyk *et al.*, 2004).

GENETIC MANIPULATIONS RESULTING IN CHANGES OF ANGE INTENSITY

In most cases of genetic manipulation having an impact on ANGE, this influence is limited. Such is the case of mice with mutated genes encoding gaseous second messenger synthases, growth-factors, membrane receptors, cyclin inhibitor, and transcription factors. Proteins encoded by these genes are usually known to regulate cell proliferation but also other processes like apoptosis.

NITRIC OXIDE SYNTHASES

Nitric oxide (NO) acts as a signaling molecule and an important negative regulator of cell proliferation in the adult mammalian brain. NO is synthesized by three different isoforms of NO synthase (NOS): nNOS (neuronal), eNOS (endothelial) and iNOS (inducible). Effects of deleting NOS genes have been reviewed (Huang, 1999; 2000; Kawashima & Yokoyama, 2004). The effect of NOS deletion on ANGE seems to be isoform-dependent.

nNOS is the enzyme responsible for producing the majority of NO in the adult mammalian brain (Huang *et al.*, 1993). nNOS expression colocalizes with ANGE and pharmacological inhibition of nNOS increases ANGE (Packer *et al.*, 2003; Sun *et al.*, 2005). Deleting nNOS in KO mice (Packer *et al.*, 2003) strongly augmented the number of new cells generated in SVZ (24% increase), rostral migratory stream (42%), OB (20%) and DG (33%).

Similarly, other nNOS KO mice show reduced infarct size and increased neurogenesis, both basal and ischemia-induced (Sun *et al.*, 2005). These mice display grossly normal appearance, locomotor activity, breeding, long-term depression and long-term potentiation (LTP), and are resistant to neural stroke. However, they show a deficit in Morris water maze, a large increase in aggressive behavior and excess, inappropriate sexual behavior (Kirchner *et al.*, 2004; Nelson *et al.*, 1995).

On the other hand, eNOS-deficient mice show a significant reduction in neuronal progenitor cell proliferation in DG (Reif *et al.*, 2004). These mice have decreased SVZ progenitor cell proliferation and migration following stroke (Chen *et al.*, 2005). eNOS KO mice have defects in the production of hematopoietic and endothelial progenitors as well (Aicher *et al.*, 2003).

In iNOS KO compared with wild-type mice, the number of dividing cells in DG was reduced ipsilaterally to an ischemic lesion, pointing to iNOS as a positive mediator of ischemia-induced (but not basal) neurogenesis (Zhu *et al.*, 2003).

GROWTH FACTORS

BDNF, brain-derived neurotrophic factor participates in synaptic plasticity and the adaptive changes in the strength of communication between neurons thought to underlie aspects of behavioral adaptation. It is widely expressed in the developing and adult brain (Kernie *et al.*, 2000; Conner *et al.*, 1997) and is essential for the differentiation and survival of many populations of neurons during development (Ip *et al.*, 1993; Cheng & Mattson, 1994; Lindholm *et al.*, 1996; Linnarsson *et al.*, 2000). Finally, BDNF promotes ANGE (Benraiss *et al.*, 2001; Pencea *et al.*, 2001).

BDNF KO mutations confer severe neurological dysfunction on newborn pups, resulting in early

death (Conover *et al.*, 1995; Liebl *et al.*, 1997). Therefore either heterozygous (Lyons *et al.*, 1999; Chourbaji *et al.*, 2004) or inducible/restricted (Gorski *et al.*, 2003; Baquet *et al.*, 2004; Monteggia *et al.*, 2004) systems were used in subsequent studies.

Heterozygous BDNF+/– mice have a normal life span. These animals develop enhanced intermale aggressiveness and hyperphagia accompanied by significant weight gain in early adulthood (Lyons *et al.*, 1999), while others report that BDNF+/– mice are indistinguishable from wild-type littermates in locomotor activity, exploration, anxiety, fear-associated learning, and behavioral despair (Chourbaji *et al.*, 2004). The newly generated neurons in DG contain BDNF. The number of dividing cells is reduced in DG of BDNF+/– mice. This reduction is associated with a significant decrease in DG volume (Lee *et al.*, 2002).

Basic fibroblast growth factor (bFGF, Fgf2), a neurotrophic factor, is involved in the development, maintenance, and survival/regeneration of the nervous system. It is broadly expressed in numerous precursor populations exhibiting spatiotemporal regulation during ontogeny (Powell *et al.*, 1991; Riva & Mocchetti, 1991; Fayein *et al.*, 1992; Ozawa *et al.*, 1997; for a review, see Reuss & von Bohlen und Halbach, 2003).

Fgf2 KO are morphologically normal, viable and fertile, however, they display decreased vascular smooth muscle contractility, low blood pressure and thrombocytosis (Dono *et al.*, 1998; Zhou *et al.*, 1998). They also demonstrate mild cardiovascular and skeletal alterations, a significant reduction in the number of cortical neurons and disturbed cortical layering while neuronal cell density is normal in the striatum and the hippocampus (Dono *et al.*, 1998; Ortega *et al.*, 1998). Moreover, astrocytes of Fgf2 KO mice show drastically reduced glial fibrillary acidic protein (GFAP) in gray, but not white matter of the forebrain (Reuss *et al.*, 2003). Possibly as a consequence of these changes mice have a leaky blood–brain barrier. Finally, Fgf2 KO mice show enlarged infarct volume and loss of brain-derived neurotrophic factor (BDNF) mRNA induction following brain ischemia (Kiprianova *et al.*, 2004).

Roles for Fgf2 in regulating neuronal production are suggested by patterns of ligand/receptor expression and proliferative effects in cultured precursors, including those from prenatal cerebral cortex, postnatal cerebellum as well as neonatal and adult hippocampal and SVZ formation (Gensburger *et al.*, 1987; Gao *et al.*, 1991; Riva & Mocchetti, 1991; Wanaka *et al.*, 1991; Ray *et al.*, 1993; Tao *et al.*, 1996; Ozawa *et al.*, 1997; Gritti *et al.*, 1999; Palmer *et al.*, 1999).

Fgf2 and Fgf receptor (FgfR) proteins are expressed within ANGE areas. Moreover, environmental levels of Fgf2 regulate neonatal hippocampal neurogenesis (Cheng *et al.*, 2002) with distinct, stage-specific roles of Fgf2 in the DG granule cell lineage. In Fgf2 KO mice there is a 30% decrease in DG neuron number at P21. Adult Fgf2 KO have a 50% reduction in SVZ dividing progenitors without changing their cell cycle time. As a result, Fgf2 KO mice have smaller OB (Zheng *et al.*, 2004).

Insulin-like growth factor 1 (IGF1) is a polypeptide related to insulin, synthesized locally in many tissues, including the brain, where it is highly expressed and is essential for normal brain development. IGF1 promotes neuronal survival (mainly in the hippocampal and olfactory systems), projection neuron growth, dendritic arborization, synaptogenesis as well as glucose utilization (reviewed in Bondy & Cheng, 2004).

IGF1 KO mice show strongly reduced perinatal survival (<5%) and dwarfism (Baker *et al.*, 1993; Liu *et al.*, 1993; Powell-Braxton *et al.*, 1993). Adult IGF1 KO have reduced brain weights, with reductions evenly affecting all major brain areas apart from DG granule cell layer volume that is reduced in excess (Beck *et al.*, 1995). The proliferation of DG progenitors in IGF1 KO appears to be enhanced, as shown by increased cell numbers and increased cell proliferation in the IGF1 KO SGZ. The incidence of apoptosis is also increased, however, suggesting that impaired survival rather than impaired proliferation accounts for the reduction in DG granule neuron number in the IGF1 KO brain. This effect is observed in both developing and adult brain (Cheng *et al.*, 2001).

RECEPTORS

The endocannabinoid system consists of a small family of endogenous ligands, ligand receptors, and ligand-metabolizing enzymes. Endocannabinoids are defined as endogenous cannabimimetic compounds capable of binding to and functionally activating cannabinoid (CB) receptors (reviewed in McPartland, 2004). Two known CB receptors are metabotropic G-protein-coupled receptors. CB1 cannabinoid receptor (CB1R) predominates in CNS, whereas CB2 is largely restricted to cells of immune function (Felder & Glass, 1998).

VR1 vanilloid receptor (transient receptor potential vanilloid channel 1, capsaicin receptor), an ionotropic cation channel, also signals as an endocannabinoid receptor (Zygmunt *et al.*, 1999). Heterologously expressed VR1 can be activated by vanilloid compounds, protons, or heat *in vitro* (Caterina *et al.*, 2000). VR1 antagonists inhibit the proapoptotic effect of VR1 receptor activation in neuroblastoma and lymphoma cells (Maccarrone *et al.*, 2000).

Endogenous cannabinoid signaling pathways have been implicated in a broad range of physiological functions, including memory and survival after

brain injury (Jin *et al.*, 2000; Mechoulam *et al.*, 2002). In addition, cannabinergic systems may also have an important role in brain development, possibly by influencing the expression of the Bcl-2/Bax system (Fernandez-Ruiz *et al.*, 2000). Finally, cannabinoids promote the survival of oligodendrocyte progenitors (Molina-Holgado *et al.*, 2002).

CB1R KO mice do not show effects of cannabinoid drugs, i.e., analgesia, reinforcement, hypothermia, hypolocomotion, and hypotension. These mice present a mild impairment in the adaptation to new environment (Ledent *et al.*, 1999). CB1R KO reduced ANGE by 50% in DG and SVZ. Moreover, dividing cells in SGZ and SVZ of WT mice expressed CB1R (Morales & Backman, 2002; Jin *et al.*, 2004).

VR1 KO mice are viable, fertile, with no differences in general appearance, gross anatomy, body weight, locomotion, or overt behavior. These mice show normal responses to noxious mechanical stimuli but exhibit no vanilloid-evoked pain behavior, are impaired in the detection of painful heat, and demonstrate little thermal hypersensitivity in the setting of inflammation (Caterina *et al.*, 2000). Jin *et al.* (2004) showed that blockade of VR1 promotes ANGE in DG and SVZ.

Gastric inhibitory polypeptide (GIP), a glucose-dependent insulinotropic polypeptide (42 aa), is a member of the vasoactive intestinal peptide-VIP/secretin/glucagon family of gastrointestinal regulatory polypeptides. GIP was found to be mitogenic in several cell types (summarized in Nyberg *et al.*, 2005). Expression of the gastric inhibitory polypeptide receptor (GIPR) gene and GIP binding sites have been described in the adult brain, including the hippocampus (Kaplan & Vigna, 1994; Usdin *et al.*, 1993).

GIPR KO mice show no gross abnormalities in general behavior, feeding, body weight and no histological abnormalities, though they have higher blood glucose levels with impaired initial insulin response after oral glucose load and even after high-fat diet (Miyawaki *et al.*, 1999). Nyberg *et al.* (2005) observed that GIPR KO mice produce significantly fewer cells in the adult granule cell layer of DG compared with wild-type mice. Also, exogenously delivered GIP induced proliferation of adult-derived hippocampal progenitors *in vivo* as well as *in vitro*.

Corticosteroids act *via* intracellular receptors that recognize specific palindromic DNA sequences in the promoter region of target genes and thereby modulate transcription. Two receptor subtypes are effective in the brain: mineralocorticoid receptor (MR; type 1) and glucocorticoid receptor (GR; type 2) (Beato *et al.*, 1995).

When untreated, MR KO mice develop pseudohypoaldosteronism after birth and die due to severe renal loss of sodium and water (Berger *et al.*, 1998). MR KO animals, however, can be rescued by exogenous NaCl administration and subsequently studied during adulthood (Bleich *et al.*, 1999). Since most mice with overall disruption of the GR gene die perinatally due to respiratory failure (Cole *et al.*, 1995), the role of GR was studied in GRNesCre mice. GRNesCre mice have a brain-specific disruption of the GR gene using the Cre/loxP-recombination system, with the Cre recombinase under the control of the rat nestin promoter, which inactivates the GR gene early during development in neuronal and glial cell precursors (Tronche *et al.*, 1999). Neuropathological analyses revealed changes in the hippocampus of adult NaCl-rescued MR KO mice but not in GRNesCre mice. Outside the hippocampus, neither MR KO nor GRNesCre mice exhibited any neuropathological changes. Finally, corticosteroid receptor mutant mice show alterations in their emotional behavior (Urani & Gass, 2003).

Adult MR KO mice demonstrate a significant reduction of granule cell neurogenesis to 65% of wild-type littermates. Interestingly, at postnatal day 8 no difference in granule cell proliferation could be demonstrated between MR KO and wild-type mice. Neurogenesis was undisturbed in adult GRNesCre mice, indicating that the basal rate of granule cell proliferation does not depend on corticosterone-evoked signaling mediated by GR and attributing long-term trophic effects of adrenal steroids on DG cells to MR (Gass *et al.*, 2000).

The neurokinin-1 receptor (NK1R) is the preferred receptor for the neuropeptide substance P (SP). SP and NK1Rs have a role in the pathophysiology of depression and/or anxiety disorders. Administered SP, *via* NK1R, can have memory-promoting, reinforcing and anxiolytic-like effects when administered systemically or into the nucleus basalis of the ventral pallidum (Hasenohrl *et al.*, 2000).

NK1R KO mice are remarkably similar both behaviorally and neurochemically to mice and other rodents treated chronically with established antidepressants (Rupniak *et al.*, 2001), i.e., KO mice are comparable to antidepressant-treated mice in neonatal separation, tail suspension, resident-intruder and forced-swim tests, assays which monitor stress responses relevant to anxiety and depression and which are widely used to test the efficacy of antidepressant drugs (De Felipe *et al.*, 1998; Rupniak *et al.*, 2000; 2001; Santarelli *et al.*, 2001). NK1R KO mice are normal in hippocampus-dependent fear conditioning and they display a mild improvement in spatial learning in the water maze task (Morcuende *et al.*, 2003).

Adult NK1R KO mice showed 29.3% enhancement in ANGE, mostly in SGZ. This increase, however, was not accompanied by an increase in cell survival since the enhancement was observed 1 day following production of marked cells but was gone 7, 14 or 28 days later. This suggests that there is a period of rapid cell death in NK1R KO mice be-

tween 1 and 7 days after production. Finally, chronic treatment with antidepressants (despiramine or citalopram) increased ANGE in wild-type mice but failed to increase the number of newborn cells in the NK1R KO animals (Morcuende *et al.*, 2003).

mCD24 (mouse Cluster and Differentiation 24/heat stable antigen – HSA, p31, nectadrin), a glycosylphosphatidylinositol-anchored molecule, is a membrane associated, highly glycosylated, 30-aa peptide that probably transduces signals in a range of cell types using protein tyrosine kinases (Stefanova *et al.*, 1991).

It is expressed in differentiating neurons during development (Kuchler *et al.*, 1989; Nedelec *et al.*, 1992; Shirasawa *et al.*, 1993) where it acts as an inhibitor of neurite outgrowth and cell proliferation (Shewan *et al.*, 1996; Nieoullon *et al.*, 2005). mCD24 is also found in ANGE zones (Calaora *et al.*, 1996; Chazal *et al.*, 2000).

mCD24 KO mice show a leaky block in lymphocytes B development and altered erythrocytes (Nielsen *et al.*, 1997). In the adult brain, these mice reveal an increase in both rapid (in SVZ and DG) and slow (SVZ) proliferating cells together with a global reduction of cell cycle duration of rapidly proliferating precursors and increased apoptosis in SVZ (Belvindrah *et al.*, 2002).

TRANSCRIPTION FACTORS

High-mobility-group (HMG) proteins bind DNA non-sequence-specifically, but specifically recognize DNA structures. These small proteins can enhance the structural flexibility of DNA influencing various processes such as transcription and recombination (Grasser, 2003). Sox2, with Sox1 and Sox3, are members of the SRY-related HMG box gene family encoding transcription factors with a single HMG DNA-binding domain, regulating crucial developmental decisions in different systems (Kamachi *et al.*, 2000).

Sox2 is expressed in embryonic neural stem cells, it is expressed in, and is essential for, totipotent inner cell mass stem cells and other early multipotent cell lineages, and its ablation in Sox2 KO mice ($Sox2^{\beta\text{-geo}}$ 'knock-in') causes early embryonic lethality shortly after implantation (Avilion *et al.*, 2003; Zappone *et al.*, 2000). In the adult, Sox2 is expressed in the vast majority of dividing precursors in the neurogenic regions: SVZ, rostral migratory stream and SGZ (Ferri *et al.*, 2004; Komitova & Eriksson, 2004).

Compound $Sox2^{\beta\text{-geo}/\Delta ENH}$ heterozygotes with a regulatory mutant allele ($Sox2^{\Delta ENH}$), in which a neural cell-specific enhancer (Zappone *et al.*, 2000) is deleted, are born in reduced numbers compared with the expected frequency, and their number further decline in postnatal life. They show growth retardation, normally compensated by six weeks of age, slowed reactivity, feet-clasping phenotype, circling behavior and epilepsy. Moreover, they have important cerebral malformations, with parenchymal loss and ventricle enlargement, degeneration, and cytoplasmic protein aggregates observed in thalamus, striatum and septum neurons. Finally, precursor cell proliferation and the generation of new neurons in SGZ (about 65% reduction) and in SVZ (~55% reduction) are decreased while GFAP/nestin-positive hippocampal cells are strikingly diminished (Ferri *et al.*, 2004).

OTHER PROTEINS

Cyclin-dependent kinase inhibitor p27Kip1, together with p21Waf1 and p57Kip2 is a member of the Kip (Cip) family of proteins that act as negative regulators of G1 cyclin-dependent kinases (G1 CDKs) affecting the duration of the G1 phase of the cell cycle (Sherr & Roberts, 1995). P27Kip1 is expressed in the SVZ (van Lookeren Campagne & Gill, 1998) and regulates the length of the G1 phase of the cell cycle in embryonic CNS progenitors (Mitsuhashi *et al.*, 2001).

p27Kip1 KO mice grow to a greater size than controls. Mutant female mice are infertile (Kiyokawa *et al.*, 1996). p27Kip1 KO mice show impaired exit from the cell cycle of glial progenitors, as a defective growth arrest was observed for both oligodendrocytes and astrocytes resulting in expanded pools of glial cells in the cortex and in the cerebellum (Casaccia-Bonnefil *et al.*, 1997; 1999).

Deleting p27Kip1 has very specific effects on a population of CNS progenitors responsible for ANGE in SVZ (Doetsch *et al.*, 2002). Loss of p27Kip1 has no effect on the number of stem cells but results in a selective increase of the transit-amplifying type C cells, decrease in the number of type A neuroblasts, and increased apoptosis. Therefore, the role of p27Kip1 is not equivalent in the different cell populations of SVZ, and the cell-cycle regulation of SVZ adult progenitors is remarkably cell-type specific. p27Kip1 appears to be a key regulator of the cell division of the transit-amplifying progenitors.

The Sanfilippo syndrome type B (mucopolysaccharidosis III B, MPS III B) is an autosomal recessive disorder caused by a lack of activity of α-*N*-acetylglucosaminidase (Naglu), one of the lysosomal enzymes needed to degrade heparan sulfate, and the resulting accumulation of this glycosaminoglycan. In MPS III B, CNS is particularly affected with progressive mental retardation accompanied by intense hyperactivity and early death (Li *et al.*, 2002).

Naglu KO mice (Li *et al.*, 1999) are healthy and fertile while young and can survive for 8–12 months, although they show vacuolation in many

cells, including macrophages, epithelial cells, and neurons. Surprisingly, Naglu KO mice manifest abnormal hypoactive behavior in an open field test (hyperactivity is not observed) and show normal response to fear conditioning test. ANGE in SVZ is inhibited in Naglu KO mouse brain at both young and adult ages, while ANGE in SGZ is decreased in 6-month-old but not in 3-month-old Naglu KO mice (Li *et al.*, 2002).

Cystatins constitute a superfamily of cysteine protease inhibitors (Barrett *et al.*, 1986). Their altered activities have been implicated in human disorders such as cancer, rheumatoid arthritis, sepsis, and osteoporosis. Cystatin C (a major mammalian cysteine protease inhibitor) is a potent inhibitor of cathepsins. It is a secreted protein composed of 120aa (Abrahamson *et al.*, 1990; Huh *et al.*, 1995). Cystatin C is present in virtually all mammalian tissues, although the inhibitor is found at particularly high concentrations in the cerebrospinal fluid of CNS (Lofberg & Grubb, 1979).

Cystatin C KO mice are fertile and show no gross pathological abnormality. They also show decreased metastatic spread (Huh *et al.*, 1999). In cystatin C KO mice the level of dividing cells in the SGZ was decreased as compared to wild-type littermates. Interestingly, the migration of newly born cells to the upper parts of the DG granule cell layer following *status epilepticus* was decreased in cystatin C KO mice (Pirttila *et al.*, 2004).

Programmed cell death in the adult brain plays a significant role in the regulation of multiple aspects of ANGE. Bax (Oltvai *et al.*, 1993), a proapoptotic member of the Bcl-2 family (reviewed in Heiser *et al.*, 2004), is essential for programmed cell death (Lindsten *et al.*, 2000), e.g., it mediates target-dependent apoptosis of neurons during embryonic development (Deckwerth *et al.*, 1996; White *et al.*, 1998; Sun *et al.*, 2003; Sun & Oppenheim, 2003). Bax normally resides in the cytoplasm, but translocates to the outer mitochondrial membrane during apoptosis. Once associated with mitochondria, Bax causes a release of apoptogenic factors from the mitochondria into the cytoplasm (Kirkland & Franklin, 2003).

Bax KO mice do not exhibit developmental apoptosis of dorsal root ganglion sensory neurons, superior cervical ganglion sympathetic neurons, or motoneurons (Deckwerth *et al.*, 1996; Lentz *et al.*, 1999; White *et al.*, 1998; Sun *et al.*, 2003). Apoptosis in the adult hippocampus is also virtually absent or greatly reduced in Bax KO mice since the number of marked (dividing) cells was virtually the same in their SGZ after one month, compared with 70% reduction of these cells in wild-type mice. The Bax-dependent pathway appears essential for apoptosis of adult-generated hippocampal neurons (Sun *et al.*, 2004).

GENETIC DEFICITS AFFECTING ANGE ONLY IN SPECIFIC CONDITIONS

In several reports, genetic model organisms show alterations in ANGE only in specific experimental situations, i.e., their constitutive ANGE is usually not changed whereas the inducible ANGE is altered. Some cases of altered inducible ANGE were already mentioned (iNOS, NK1R, and cystatin C KO mice).

Presenilin-1 (PS1) is a polytopic membrane protein which plays a critical role in facilitating intramembranous processing of Notch, a signaling receptor that is essential for neuronal fate specification and differentiation. Presenilins are expressed during neuronal development and are present in neuronal cells in the hippocampus and the cortex (Lee *et al.*, 1996). PS1 KO in mice is associated with severe developmental abnormalities and neonatal embryonic lethality (Shen *et al.*, 1997; Wong *et al.*, 1997; Handler *et al.*, 2000), suggesting an essential role of PS1 in development.

Conditional double knockout mice lacking both presenilins in the postnatal forebrain exhibit impairments in hippocampal memory and synaptic plasticity followed by neurodegeneration (Saura *et al.*, 2004). Feng *et al.* (2004) showed that forebrain-specific PS1 (PS1 FB-KO) deletion results in reduced enrichment-induced neurogenesis in DG. The PS1 FB-KO mice show no anatomical differences in brain structure; they normally mate, grow, and exhibit normal open field behavior. The numbers of newborn cells in DG show no differences between PS1 FB-KO and control mice. However, after two weeks of exposition to enriched environment KO mice show a significantly lower level of dividing cells in DG (37% less) compared with control mice. It confirms that loss of PS1 leads to a significant deficiency in enrichment-induced neurogenesis in FB-KO mice. This defect in neurogenesis is associated with enhanced fear memory of contextual cues when the animals are subjected to enrichment between training and testing. The authors suggest that neurogenesis in adult DG may serve to clear out outdated memory traces from the hippocampus after the memory is transferred and consolidated in the cortex, thus leaving the hippocampus available for new memory processing.

Exercise induces BDNF mRNA in the hippocampus (Neeper *et al.*, 1995), and BDNF promotes ANGE (Benraiss *et al.*, 2001; Pencea *et al.*, 2001). BDNF is an activity-induced gene regulated at the transcriptional level, and these transcriptional changes are initiated by calcium increases generated through the activation of NMDA receptors or voltage-sensitive calcium channels (Shieh *et al.*, 1998; Tao *et al.*, 1998). The NMDA receptor epsilon 1 subunit is

expressed both in the dentate granule cells and pyramidal neurons in the normal mouse hippocampus (Sakimura *et al.*, 1995).

NMDAR epsilon 1 KO does not cause any detectable changes in the shape and volume in the hippocampal granule cell layer (Kitamura *et al.*, 2003). NMDAR epsilon 1 KO show significant reduction of the NMDA receptor channel current and LTP at the hippocampal CA1 synapses as well as an increase of locomotor activity in a novel environment and an impairment of spatial, contextual, and latent learning (Sakimura *et al.*, 1995; Miyamoto & Nabeshima, 2002).

There is no difference in neurogenesis in SGZ between WT and NMDAR epsilon 1 KO control mice. The hippocampal neurogenesis as well as BDNF expression are enhanced when wild-type mice are raised in cages with running wheels for 3 weeks. In NMDAR epsilon 1 KO mice, no difference in neurogenesis or BDNF levels was detected between the exercise and control groups. The exercise-induced cellular proliferation in the hippocampus, but not basal proliferation, is dependent on NMDA receptors (Kitamura *et al.*, 2003).

The cyclooxygenase (Cox) enzymes catalyze a key step in the conversion of arachidonate to prostaglandin H2, the immediate substrate for a series of cell-specific prostaglandin and thromboxane synthases. Prostaglandins play critical roles in numerous biologic processes, including the regulation of immune function, kidney development, reproductive biology, and gastrointestinal integrity. There are two isoforms: Cox-1 is constitutively expressed in most tissues and responsible for tissue homeostasis, whereas Cox-2 is usually absent, but is induced by numerous physiologic stimuli, e.g., plays an important role in inflammation and tumorigenesis (Williams *et al.*, 1999; Simmons *et al.*, 2004).

Cox-2 KO mice show reproductive anomalies and defects in kidney development (Williams *et al.*, 1999). In the postischemic DG of heterozygous and homozygous Cox-2 KO mice, proliferating cells were significantly fewer than in wild-type littermates (Sasaki *et al.*, 2003).

Among the 14 known serotonin (5-HT) receptor subtypes, the 5-HT1A receptor (5-HT1AR) has been implicated in the modulation of mood and anxiety-related behaviors (Buhot, 1997; Hoyer *et al.*, 1994; Menard & Treit, 1999). Brain 5-HT1ARs are located pre- and postsynaptically. Presynaptic 5-HT1ARs are found in the dorsal and median raphe nuclei and serve to negatively regulate serotonergic cell firing. Postsynaptic 5-HT1ARs are found on nonserotonergic neurons in limbic regions (e.g., hippocampus, septum, cerebral cortex and amygdala). Activation of postsynaptic 5-HT1AR is believed to induce a decrease in the firing rate of the postsynaptic cell (Pazos & Palacios, 1985; Blier *et al.*, 1987; Sprouse & Aghajanian, 1988; Pompeiano *et al.*, 1992;). Agonists of 5-HT1AR have anxiolytic properties both in humans and in animal models (Feighner & Boyer, 1989; Barrett & Vanover, 1993; Lucki *et al.*, 1994; De Vry, 1995; Menard & Treit, 1999).

5-HT1AR KO mice have normal growth and viability and do not display any obvious morphological or behavioral abnormalities as well as no abnormalities of serotonin system development. However, these mice show increased anxiety-like behavior in a variety of tests (Heisler *et al.*, 1998; Parks *et al.*, 1998; Ramboz *et al.*, 1998; Gross *et al.*, 2002; reviewed in: Zhuang *et al.*, 1999; Gross *et al.*, 2000; Groenink *et al.*, 2003a; 2003b; Overstreet *et al.*, 2003). Additionally, in the hippocampal-dependent spatial reference memory version of the Morris water maze young-adult 5-HT1A KO mice exhibit impairment in learning and retention of the spatial task. This genotype effect does not persist during aging. In fact, aged 5-HT1A KO mice seem to be slightly facilitated during the early stages of learning (Wolff *et al.*, 2004).

Administration of fluoxetine, an antidepressant, causes a doubling of the number of dividing cells in wild-type mice but has no effect in 5-HT1AR KO mice, while chronic treatment with another antidepressant, imipramine, induces neurogenesis in both DG of the wild-type and 5-HT1AR KO mice. These results indicate that 5-HT 1A receptors are required for fluoxetine-induced but not imipramine-induced neurogenesis (Santarelli *et al.*, 2003).

CONCLUSION

Transgenic mice have become an increasingly important tool used to investigate neurogenesis. Their application in research has already allowed revealing a number of genes whose protein products play an important role in ANGE and more of those are expected to come. Furthermore, it is predictable that the field of identifying novel genes and proteins pivotal for ANGE will grow rapidly in the nearest future. Combination of genetic and other molecular and cell biology approaches is a prerequisite for this rapid development.

One of the most important recent discoveries in the field was to reveal genes and proteins that are indispensable for the neurogenesis in the adult brain but not during the brain development. Hence, these results open a new avenue of research on targeting these proteins in search of specific activators and inhibitors of ANGE to develop treatment of various brain diseases. Possible involvement of ANGE has been proposed in neurodegenerative diseases, stroke, brain injury, gliomas, etc.

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