The Roles of Epigenetic Modifications in Embryonic and Adult Neurogenesis

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Katarina Vagaja

ULOGE EPIGENETSKIH MODIFIKACIJA U EMBRIONALNOJ I ADULTNOJ NEUROGENEZI

Završni rad

Rijeka, srpanj 2020.

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SUMMARY

The entire nervous system develops from common neural stem cells (NSCs) that line the neural tube during the first weeks of embryonic development. NSCs are self-renewing, multipotent cells that generate neural progenitor cells by asymmetric division, and which, through a limited number of divisions, differentiate into specialized cells of the nervous system: neurons, oligodendrocytes, and astrocytes. Although most neurons are generated before birth, it has been shown that under the influence of appropriate stimuli, in adult mammals and humans, neurogenesis continues throughout life in limited regions of the brain; the subgranular zone of the dentate gyrus in the hippocampus and the subventricular zone of the lateral ventricles. Generation of neurons from NSCs encompasses successive stages of progenitor expansion, differentiation, maturation, and integration of new-born neurons into functional neural circuits. Numerous intrinsic and extrinsic factors are involved in the regulation of neurogenesis, forming a complex network of signalling pathways. It seems that epigenetic modifications and their cooperation play an integral role in the precise control of gene expression, which is crucial for the proper acquisition of neural fate. This review will comprehensively summarize the hitherto known roles of epigenetic modifications, in particular DNA modifications, histone modifications and non-coding RNA molecules, in regulating both embryonic and adult neurogenesis in the vertebrate CNS, as well as their mutual mechanisms of cooperation. Further elucidation of the role of epigenetic mechanisms in the process of neurogenesis could lead to the development of novel therapies needed for the treatment of neurodevelopmental and neurodegenerative disorders.

KEYWORDS

Neurogenesis; epigenetics; DNA methylation; histone modifications; noncoding RNAs

SAŽETAK

Čitav živčani sustav razvija se iz zajedničkih neuralnih matičnih stanica (eng. neural stem cells, NSCs) koje oblažu neuralnu cijev tijekom prvih tjedana embrionalnog razvoja. NSCs su samoobnavljajuće, multipotentne stanice koje asimetričnom diobom generiraju neuralne progenitorske stanice, a koje kroz ograničen broj dioba diferenciraju u specijalizirane stanice živčanog sustava: neurone, oligodendrocite i astrocite. Iako većina neurona nastaje prije rođenja, dokazano je da se pod utjecajem odgovarajućih podražaja, u odraslih sisavaca i čovjeka, neurogeneza nastavlja tijekom cijeloga života u ograničenim regijama mozga; subgranularnoj zoni dentatnog gyrus-a u hipokampusu i subventrikularnoj zoni bočnih ventrikula. Stvaranje neurona iz NSCs uključuje uzastopne faze ekspanzije progenitora, diferencijaciju, sazrijevanje i integraciju novih neurona u funkcionalne neuralne krugove. U regulaciji neurogeneze sudjeluju brojni unutarnji i vanjski čimbenici, tvoreći kompleksnu mrežu signalnih puteva. Čini se da upravo epigenetske modifikacije te njihova međusobna suradnja igra sastavnu ulogu u preciznoj kontroli genske ekspresije ključnoj za pravilno usmjeravanje NSCs u stjecanje neuralne sudbine. Ovaj pregledni rad sveobuhvatno će sažeti do sad poznate uloge epigenetskih modifikacija, točnije DNA modifikacija, histonskih modifikacija i nekodirajućih RNA molekula, u regulaciji embrionalne i odrasle neurogeneze u centralnom živčanom sustavu kralježnjaka, kao i njihove međusobne mehanizme suradnje. Daljnje rasvjetljivanje uloga epigenetskih mehanizama u procesu neurogeneze mogao bi dovesti do razvoja novih terapija za liječenje neurorazvojnih i neurodegenerativnih poremećaja.

KLJUČNE RIJEČI

Neurogeneza; epigenetika; DNA metilacija; histonske modifikacije; nekodirajuće RNA molekule

GLOSSARY OF ABBREVIATIONS

5hmC	5-hydroxymethylcytosine
5mC	5-methylcytosine
aNSC	adult neural stem cell
BP	basal progenitor
CBP	CREB-binding protein
CNS	central nervous system
CX	cerebral cortex
DG	dentate gyrus
DNMT	DNA methyltransferase
ESC	embryonic stem cell
HAT	histone acetyltransferase
HDAC	histone deacetylase
HDM	histone demethylase
HMT	histone methyltransferase
IPC	intermediate progenitor cell
MBD	methyl CpG binding domain
MBP	methyl-CpG-binding protein
MeCP2	methyl-CpG-binding protein 2
ncRNA	non-coding RNA
NEC	neuroepithelial cell
NPC	neural progenitor cell
NSC	neural stem cell
OB	olfactory bulb
PRC	Polycomb repressive complex
RGC	radial glial cell
SGZ	subgranular zone
SVZ	subventricular zone
TET	ten-eleven translocation methylcytosine dioxygenase
VZ	ventricular zone

CONTENTS

1.Introduction

1.1. The Concept of Epigenetics

Epigenetics is an emerging field of genetics which studies the modifications that take place "above" the gene. Epigenetic modifications have 4 essential properties: (1) they regulate gene expression without changing the DNA sequence; (2) they are hereditary, stable through mitosis and to some extent through meiosis; (3) they are reversible, subject to change throughout life due to the influence of environmental factors; and (4) they are fundamental for cellular differentiation as they can stimulate or inhibit the activity of differentiation genes [1].

Epigenetic modifications include DNA methylation, histone modifications, and non-coding RNA molecules. The terms writers, readers, and erasers refer to key enzymes in the field of epigenetics. Writers apply covalent marks to the DNA and histone proteins. These marks are then recognized and identified by readers and they can only be removed by erasers (Figure 1) [2]. Epigenetic marks and enzymes are critical for cell fate determination of neural stem cells in both the embryonic and the adult mammalian brain.

1.1. Neurogenesis

Neurogenesis is the process by which new differentiated neurons are derived from proliferating and multipotent neural stem cells (NSCs). It is crucial during embryonic development but continues in certain regions of the brain after birth and throughout life [3].

1.1.1. Embryonic Neurogenesis

During early embryonic development, the nervous system begins to form from the ectoderm by the process of neural induction. The central event of this process is the formation of the notochord, a cylindrical rod of mesodermal cells that runs along the entire length of the developing

embryo. The notochord directs the formation of the neural plate by inducing the thickening of the ectodermal cells.

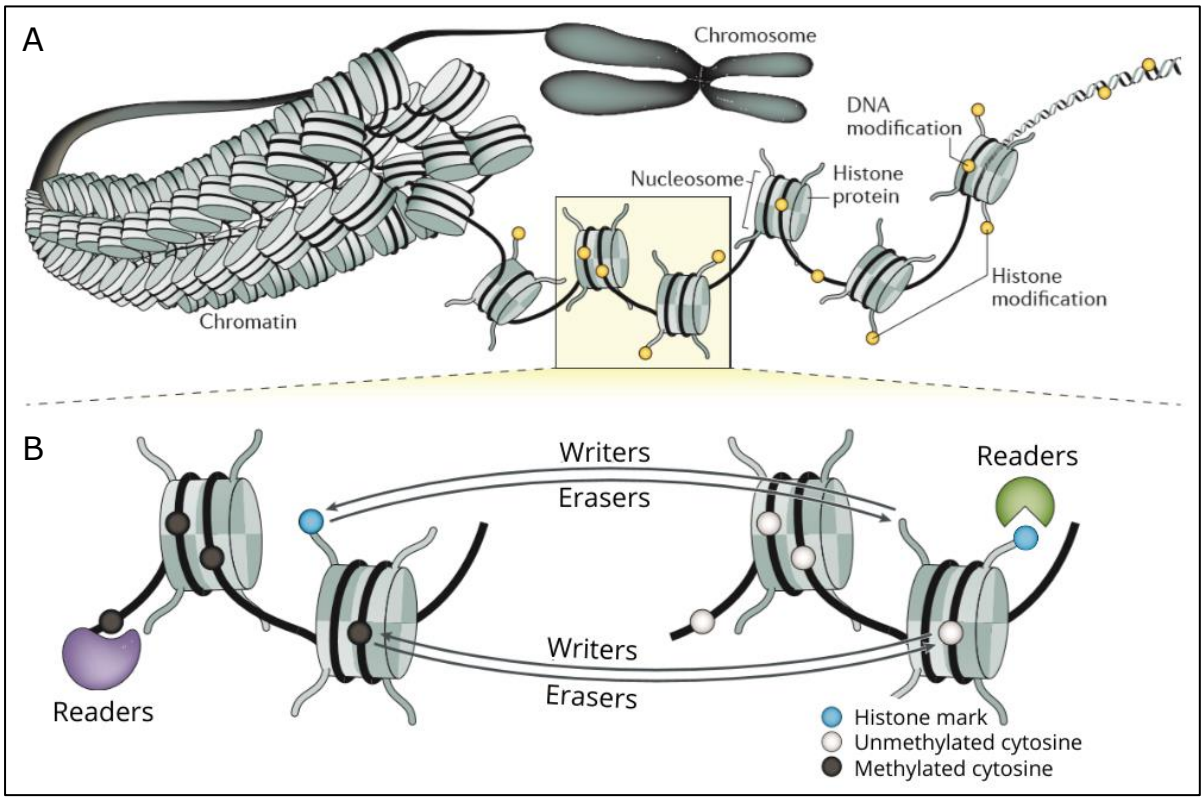

Figure 1. An overview of epigenetic modifications. A| DNA packaging in eukaryotes and covalent modifications (yellow circles) on chromatin. B| The interplay between key epigenetic enzymes; writers, readers, and erasers. (Image taken and modified from [2])

The central cells of the neural plate then move outward forming the neural groove and the neural folds. After the neural folds are brought into contact with one another, a hollow neural tube is formed (Figure 2A) [4], [5]. The early neural tube wall consists of proliferating multipotent neuroepithelial cells (NECs) that are located in the ventricular zone (VZ), the innermost cell layer surrounding the lumen of the neural tube, also known as the primary proliferative zone in the developing cerebral cortex (CX). NECs are elongated and highly polarized cells with their apical membrane facing the lumen of the neural tube and their basal membrane contacting the basal lamina. Their nuclei can be located in several separate layers, from the ventricular (apical) to the pial (basal) surface (Figure 2B), as they move depending on the cell cycle phase.

After closure of the neural tube and expansion of stem cells, NECs turn into radial glial cells (RGCs) that are considered to be the primary neural progenitor cells (NPCs) as they give rise to all neurons and glia [6], [7]. During the division cell cycle (Figure 2C), NPCs move their nuclei towards the pial surface where DNA synthesis (S phase) occurs and travel back towards the ventricular surface where mitosis (M phase) occurs [8]. Both NECs and RGCs initially divide symmetrically to expand their population in the VZ. In symmetrical mitosis the metaphase plate is perpendicular to the ventricular surface and the two identical daughter cells undergo further proliferation [9].

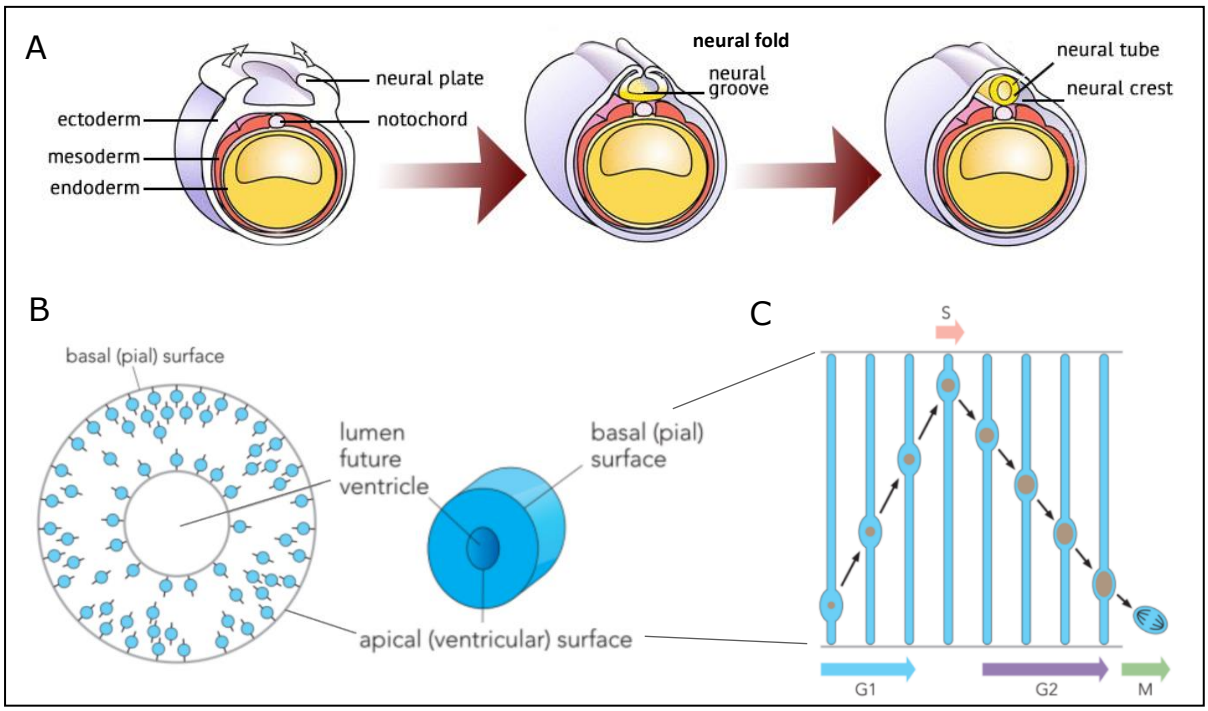

Figure 2. A| Formation of the neural tube. (Image taken from [5]) B| Cross section through the early neural tube. C| NPC division cycle. (Images taken and modified from [8])

Following stem cell proliferation, neurogenesis begins with NPCs switching from symmetric to asymmetric divisions where the metaphase plate is oriented almost parallel to the ventricular surface. Asymmetric division may produce neurons directly from RGCs (Figure 3A) or indirectly via basal progenitors (BPs) such as intermediate progenitor cells (IPCs) and basal radial glial cells (bRGCs) (Figure 3B).

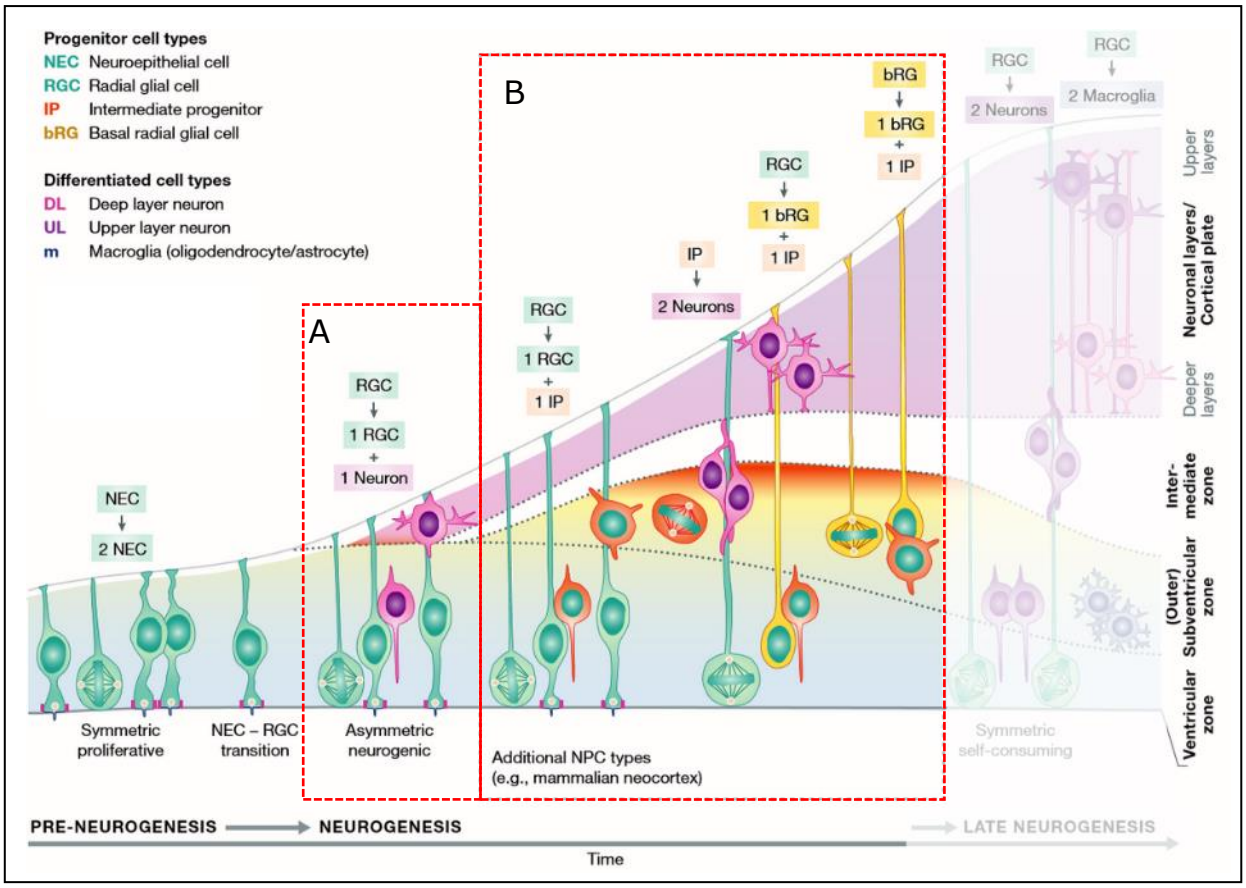

Figure 3. An overview of embryonic neurogenesis. A| Directly produced neuron. B| Indirectly produced neurons. (Image taken and modified from [7])

Neurogenesis is mostly indirect and characterized as a two-step process: (1) RG cells divide in the VZ to produce one daughter cell that remains in the proliferative state and an IP cell; and (2) IPCs migrate basally to form the second proliferative zone known as the subventricular zone (SVZ) where they symmetrically divide to produce neurons. Even though direct neurogenesis is faster, indirect neurogenesis is far more important as it results in increased neuronal numbers and cortical size [10], [6]. As the CX development progresses, the VZ disappears and the SVZ continues to proliferate giving rise to cortical neurons, neurons that will migrate to the olfactory bulb (OB), and most of the glial cells [11].

Once neurons are born, they migrate from their site of origin to their final destinations in the nervous system where they mature and ultimately generate neural circuitry. For example, neurons born in the VZ migrate radially to the cortical plate, that is neuronal layers, where they accumulate

to form the CX. Generally, neurons of similar phenotype and date of birth adopt similar fates – clustering together within specific regions of the developing nervous system [11].

1.1.2. Adult Neurogenesis

Until the 1960s, it was thought that the nervous system had no regenerative power, that is, it could not produce new nerve cells in the postnatal and adult period of life. However, it has been shown that NSCs are maintained in the adult brain of several species including humans. In most mammals, adult neurogenesis occurs in just two regions of the brain: the forebrain SVZ surrounding the lateral ventricles (LV) of the mature CX; and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) (Figure 4). There are more regions in the CNS that provide an environment or a niche that supports the maintenance of adult NSCs (aNSCs) (e.g., subventricular regions of the cerebellum, midbrain, and spinal cord), however, these stem cells do not produce new neurons *in vivo*. Therefore, only SVZ and SGZ are considered to be proliferative zones in the adult brain [4], [11], [6].

On the one hand, SVZ aNSCs produce IPCs that give rise to neuroblasts, postmitotic cells committed to becoming neurons, which undergo a maturation process by migrating through the rostral migratory stream (RMS) towards the OB where they become interneurons (Figure 5A). On the other hand, SGZ aNSCs generate IPCs that migrate locally into the inner granule cell layer of the DG where they differentiate into neuroblasts, which become immature neurons and undergo synaptic integration into the existing neural circuits (Figure 5B). Most neuroblasts die before reaching full maturity and integration into functional circuitry [11], [12], [13]. Nevertheless, this ability to recapitulate the complete process of neuronal development that these two neurogenic niches possess, plays an important role in brain plasticity, learning, memory and mood regulation [14].

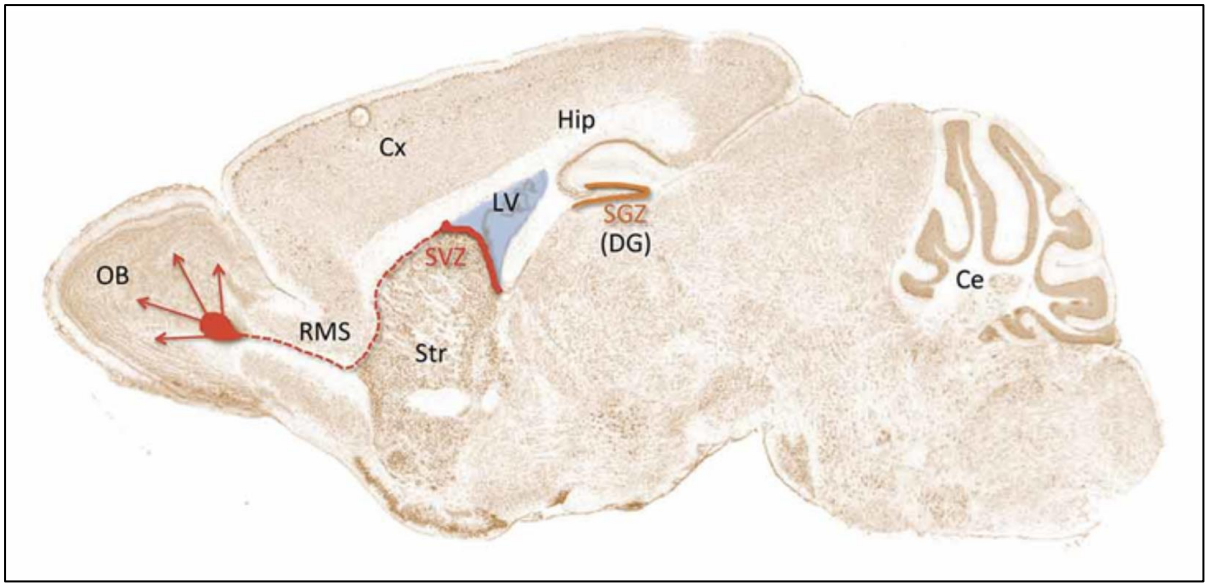

Figure 4. Two major neurogenic niches in the adult mammalian brain: the subventricular zone (SVZ) and the subgranular zone (SGZ). (Image taken from [15])

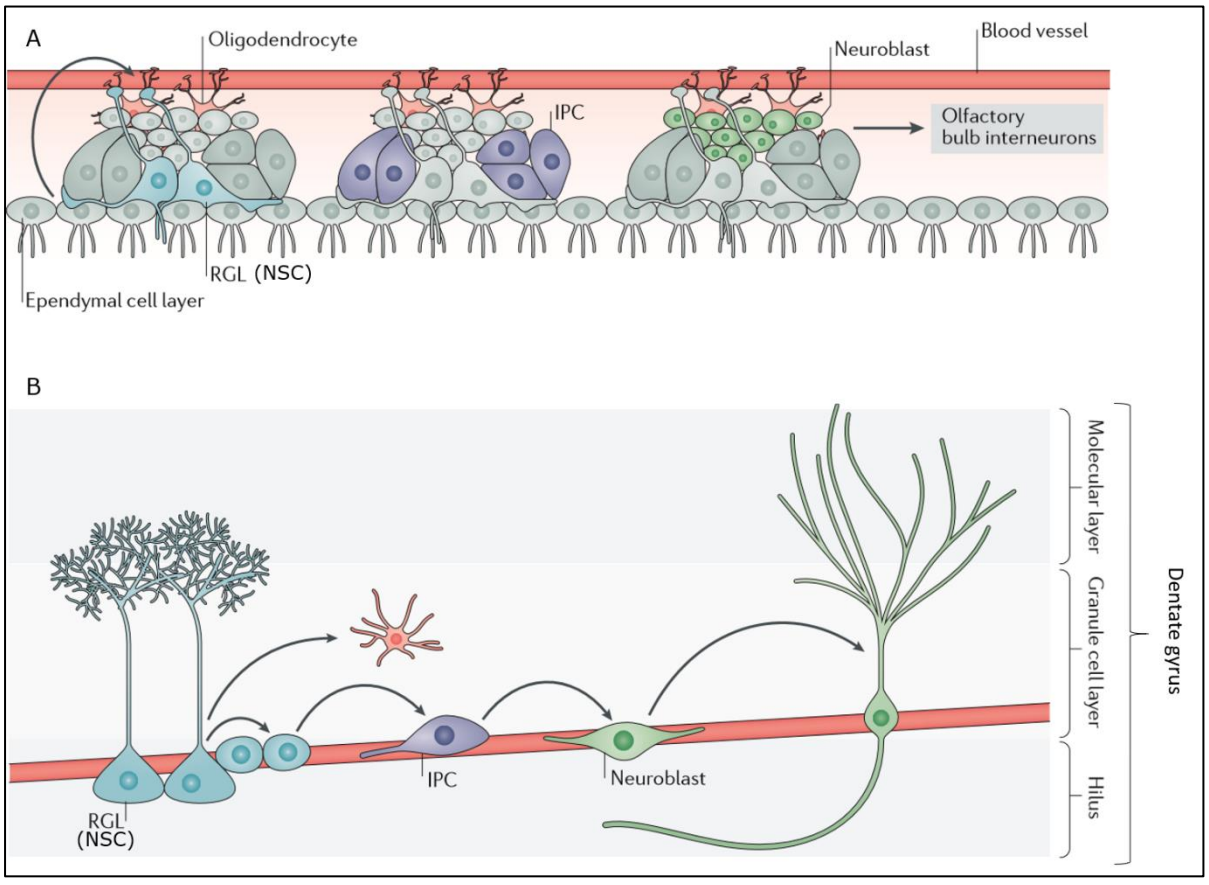

Figure 5. An overview of adult neurogenesis. A| From aNSCs in the SVZ to the OB interneurons. B| From aNSCs in the SGZ to the DG interneurons. (Image taken and modified from [16])

NSCs that reside within the DG and SVZ are activated via surrounding neurogenic niches and/or endogenous signals. A niche refers to a specific tissue microenvironment which works closely with NSCs to determine their fate. Several neurogenic factors, corresponding resident cells and blood vessels create an appropriate microenvironment necessary for proliferation, differentiation, and migration of new neuroblasts. Transcriptional factors, neurotransmitters, growth factors, cytokines, and epigenetic enzymes are only a few of the many molecular mechanisms that contribute to creating a favourable microenvironment of neurogenic niches. In addition to internal factors, many environmental factors such as exercise, stress, and antidepressants have been reported to influence the rate of adult neurogenesis [4].

1.2. Purpose of Review

Nervous system development relies on the ability of multipotent NSCs to repeatedly produce more restricted neural progenitors, which divide and generate small numbers of differentiated progeny. This process requires a wide range of signalling pathways, including complex orchestration of epigenetic mechanisms. Multiple epigenetic enzymes have been identified to regulate NSC proliferation, differentiation, and migration of neurons from their place of birth to their final location in the embryonic brain, as well as neuronal survival, maturation, and integration in the adult brain. This critical literature review will provide an insight into the key roles of DNA methylation, histone modifications and non-coding RNAs, as well as crosstalk between these mechanisms, in regulating embryonic and adult neurogenesis in the CNS. The key question this review will focus on is "What epigenetic mechanisms underlie vertebrate NSC development into neuronal progeny?"

2. DNA Methylation and Demethylation

DNA methylation is an extensively studied reversible epigenetic mechanism that regulates gene expression. For a long time it has been defined as the covalent transfer of a methyl group to 5'-carbon position of cytosine (5-methylcytosine, 5mC), however, today it is known that other positions of cytosine (e.g., N3-methylcytosine, 3mC) as well as other nucleotides (e.g., N6-methyladenine, 6mA) are also subject to methylation. Moreover, new epigenetic modifications on the C-5' position have been discovered, such as 5-hydroxymethylcytosine (5hmC), an essential intermediate of active DNA demethylation [17].

DNA methylation is catalysed by writers, a family of DNA methyltransferases (DNMTs). DNMT1 maintains methylation patterns during cell replication, while DNMT3A and DNMT3B add novel methyl groups onto unmethylated DNA. Once DNA methylation is established, a set of methyl-CpG-binding proteins (MBPs), that is readers, recognize and bind 5mC to mediate its function [16]. The best studied MBPs are a family of proteins that contain a conserved DNA binding motif – methyl-cytosine binding domain (MBD) [14]. Finally, methylation marks can be removed by a family of ten-eleven translocation methylcytosine dioxygenases (TETs), also known as erasers, which catalyse DNA demethylation, a multi-step process that involves the oxidation of 5mC into 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and ultimately 5-carboxylcytosine (5caC) which can be excised by thymine DNA glycosylase (TDG) and replaced with cytosine via base excision repair (BER) pathways (Figure 6) [2] [18].

In mammals, around 80-90% of cytosines are methylated in the context of CpG dinucleotides, while the remaining 10-20% of cytosines are found to be unmethylated in the so-called CpG islands, regions abundant with GC pairs located at promoter regions [17]. In addition to CpG methylation, cytosines which are not adjacent to guanines can be methylated, a process known as non-CpG methylation or CpH methylation, where H can be an

adenosine, a cytosine, or a thymine nucleotide. Recent studies have shown that non-CpG methylation is enriched in mouse and human brains; it seems to be more present in neuronal than non-neuronal cells; and it accumulates during postnatal development, a critical period of neuronal maturation and synaptogenesis, reaching high levels in adulthood [16], [14], [19].

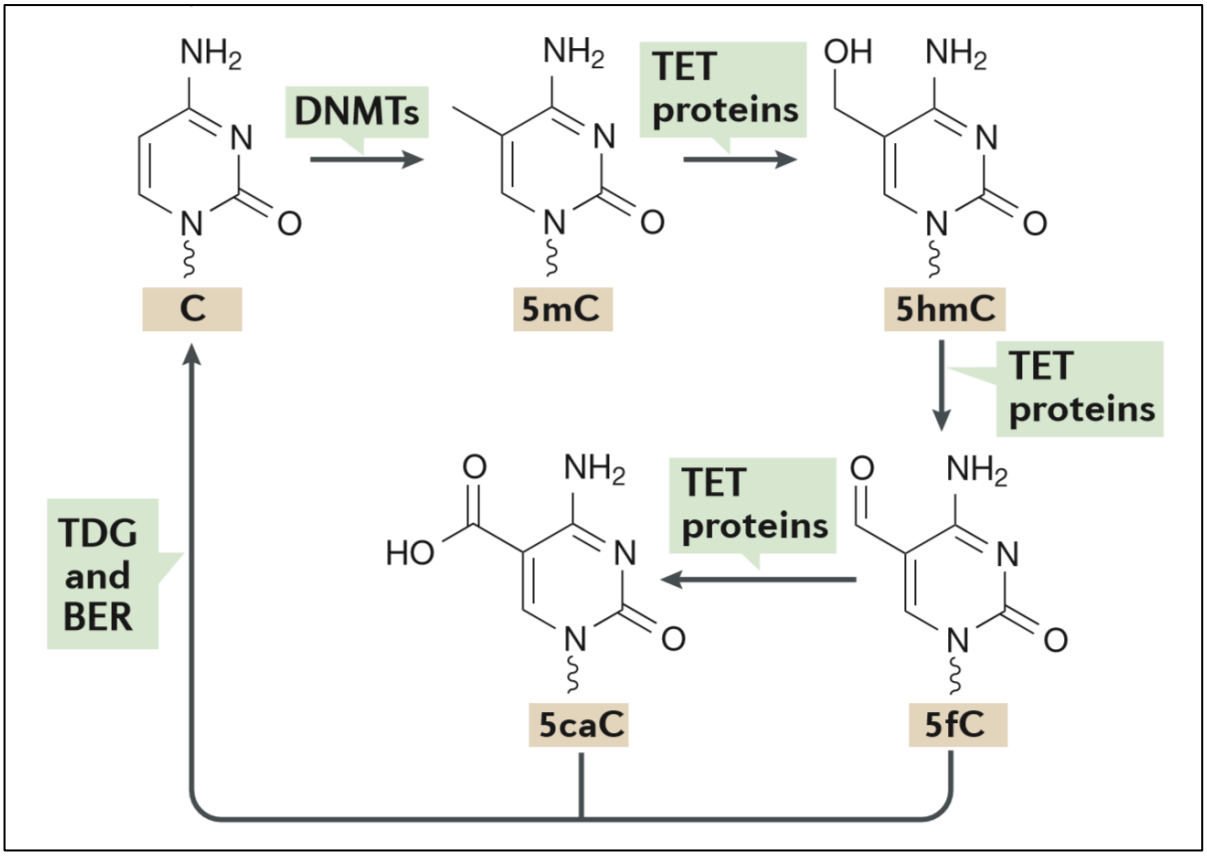

Figure 6. DNA methylation and demethylation pathways. (Image taken and modified from [16])

DNA methylation typically acts to repress gene expression by recruiting repressive histone modifiers through MBPs or by blocking the binding of transcription factors. It has an essential role in transcriptional regulation, chromatin structure, maintenance of genomic stability, genomic imprinting, X chromosome inactivation, and the establishment of cell fate [14]. The interplay between DNA methylation and demethylation enzymes has an important role in establishing and maintaining cellular identity within multiple aspects of embryonic and adult neurogenesis.

2.1. DNA (De)Methylation in Embryonic Neurogenesis

As key writers of DNA methylation, DNMTs are particularly important in embryonic neurogenesis. Its best studied family members, DNMT1, DNMT3A and DNMT3B, are expressed in different locations and during distinct stages of neurogenesis. In the embryonic mouse brain, DNMT1 is expressed in VZ NPCs and neuroblasts; DNMT3A is expressed in both VZ and SVZ NPCs from E10.5 to E17.5 and continues to be expressed in postnatal and adult neurons; and DNMT3B is expressed in the VZ from E10.5 to E13.5 but its expression ceases after E15.5 (Figure 7) [18], [16].

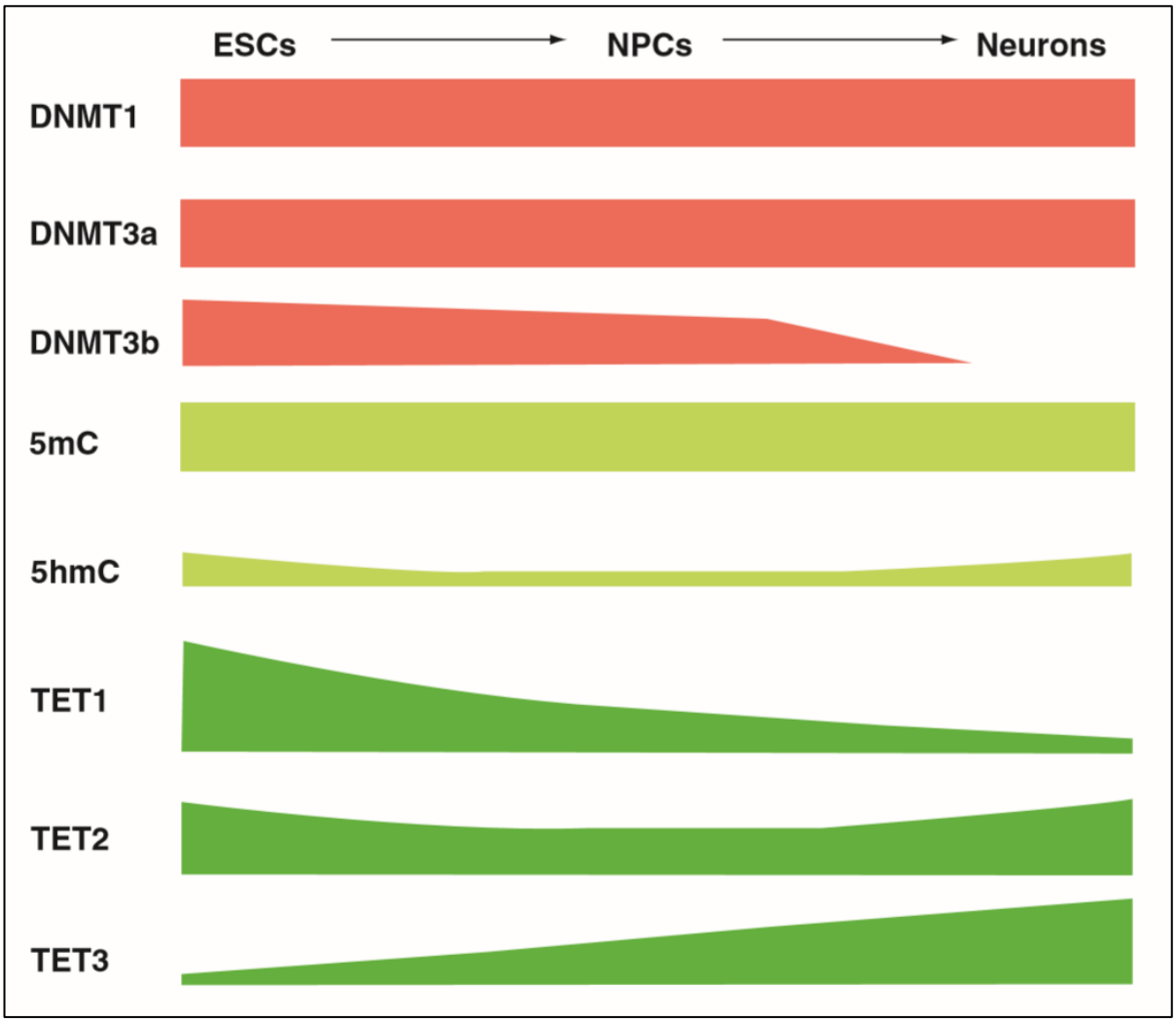

Figure 7. Spatial and temporal expression of DNMTs, TETs, 5-mC, and 5-hmC during embryonic neurogenesis. (Image taken from [18])

DNMT1 has been shown to suppress premature glial differentiation by retaining the methylation pattern of glial genes such as GFAP, as well as genes involved in the JAK-STAT pathway, which mediates the critical switch from neuro- to gliogenesis (Figure 8) [20], [21]. In the postnatal brain, DNMT3A seems to be critical for brain maturation due to *de novo* writing of non-CpG methylation which is reported to have more repressive properties than CpG methylation [16], [14]. DNMT3B appears to be critical for *de novo* methylation of certain differentiation markers in early NSCs (e.g., DLX2, NEUROG2), thereby serving to maintain their self-renewal phase [22]. Knockouts of DNMTs in mice have been reported to disrupt normal neuronal development, some causing developmental abnormalities and even lethality, highlighting their crucial roles in embryonic neurogenesis [17], [16], [18].

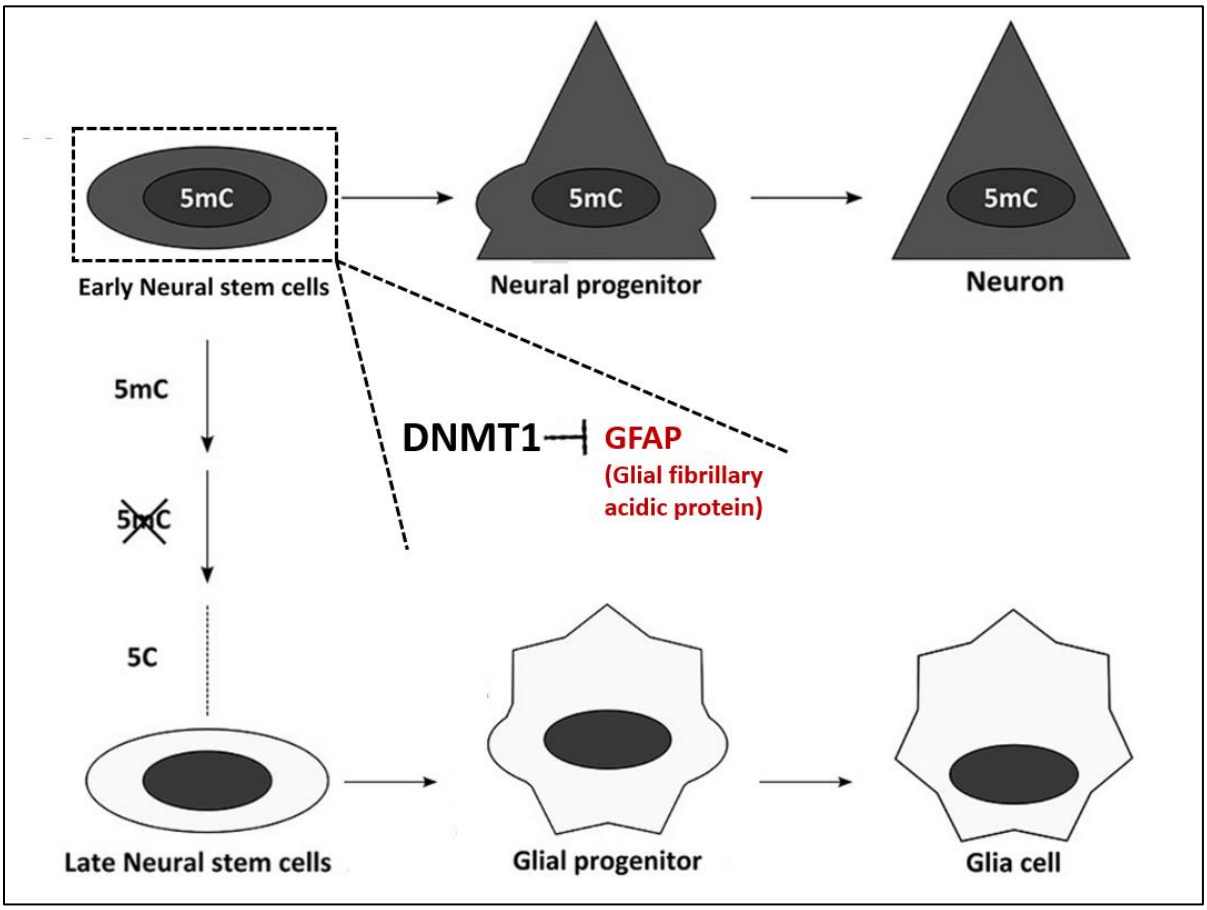

Figure 8. Suggested influences of DNA methylation in neurogenesis. (Image made from [20] and [17])

Recent studies have shed light on significant roles that key erasers of DNA methylation, TETs, have in embryonic neurogenesis. TET1 expression decreases during differentiation of mouse embryonic stem cells (ESCs) into NPCs, what correlates with approximately 80% depletion of 5hmC levels. During the same period, TET2 decreases to some extent, while TET3 increases dramatically. On the other hand, 5hmC levels increase during NPC differentiation into neurons when TET1 expression is low but TET2 and TET3 expressions are high (Figure 7) [18]. TET proteins are noted to possess dual roles in activating and repressing gene expression. By recruiting certain histone modifiers to pluripotent loci, such as the NANOG promoter, TETs generate 5hmC and promote lineage commitment of NSCs (Figure 9A). Meanwhile, TET1 is also found to mediate gene silencing by cooperating with certain transcriptional and epigenetic repressors (Figure 9B) [22].

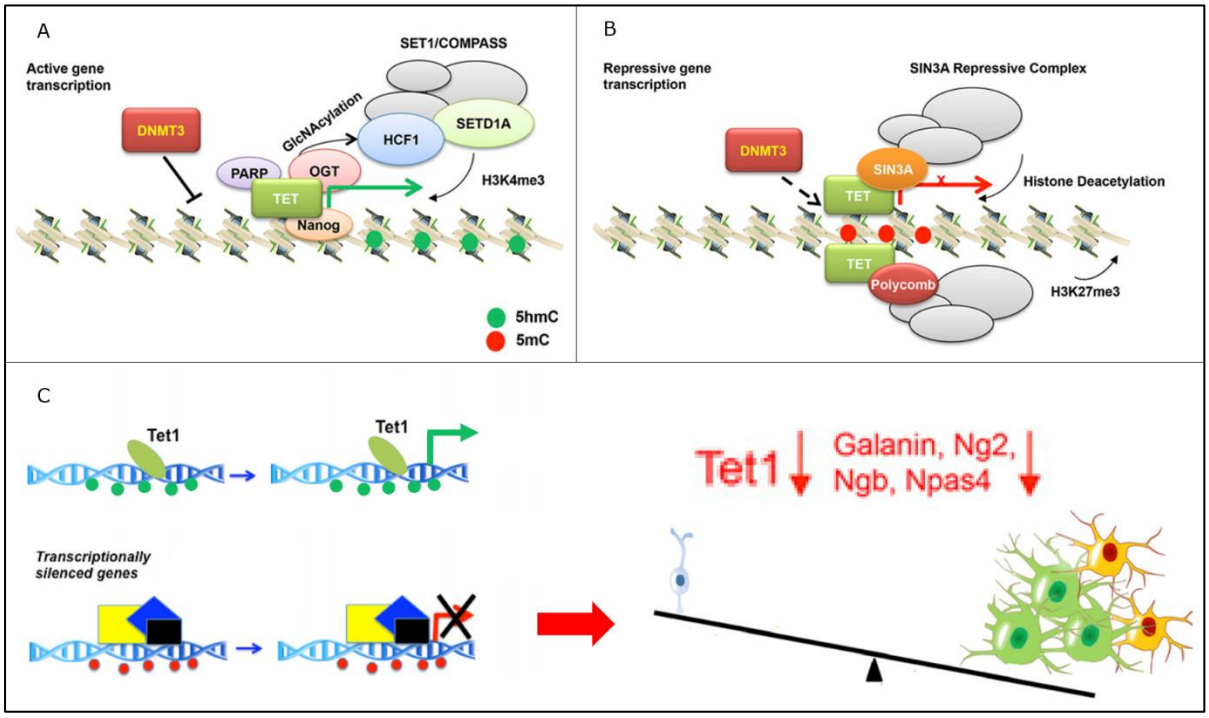

Figure 9. A| TET-mediated gene activation. B| TET-mediated gene silencing. C| The role of TET1 in adult neurogenesis. (Image made from [22], [23])

In addition to DNMTs and TETs, readers are also of vital importance in embryonic neurogenesis for their transcriptionally repressive functions. Methyl-CpG-binding protein 2 (MeCP2) has been found to act as a

transcriptional repressor and an activator depending on the context. When binding to 5-mC regions it silences gene expression by recruiting corepressors and chromatin remodelling proteins. However, 5hmC in postmitotic neurons impairs the binding of MeCP2, resulting in functional demethylation and subsequent transcriptional activation [16], [17], [24]. Full knockout of MeCP2 in mice resulted in impaired hippocampal neuronal maturation with higher expression levels of several genes related to synaptic development in the DG, highlighting its role in 5hmC-mediated demethylation [16], [18]. The conflicting role of MeCP2 in repressing and activating gene expression during neuronal differentiation remains to be elucidated.

2.2. DNA (De)Methylation in Adult Neurogenesis

Besides their major role in the developing brain, DNMTs are equally important in the adult brain. As opposed to embryonic neurogenesis where DNA methylation contributes to cell fate specification, deletion of DNMT1 in decreases the survival of newly generated neurons in the adult hippocampal DG [14]. It is suggested that DNMT1 regulates cellular survival by inhibiting the expression of pro-apoptotic genes such as PAK6 as well as genes encoding for MAP-kinases and BCL2 family members [20]. Furthermore, DNMT3A contributes to neuronal differentiation of aNSCs in the SVZ by establishing non-CpG methylation patterns in nonpromoter regions of neurogenic genes, such as DLX2, SP8 and NEUROG2, and thus inducing their expression [18], [25].

DNA (de)methylation plays a critical role in adult neurogenesis as well. One study showed that electroconvulsive therapy (ECT)-induced neuronal activation promoted DNA demethylation of certain genes, causing a sustained upregulation of hippocampal neurogenesis in adult mice [26]. DNA-damage-inducible protein 45β (GADD45β) was shown to promote active DNA demethylation, enhancing expression of critical gene promoters in DG neurons, such as BDNF and FGF1, consequently inducing aNSC proliferation and dendritic growth of new-born neurons in the adult mouse

hippocampus [18], [16]. Further studies revealed that TET1-mediated DNA demethylation in the adult brain is important for cognition, specifically learning, memory and synaptic plasticity. Deficiency of TET1 causes hypermethylation of: (1) neuronal activity-regulated genes (e.g., FOS and NPAS4), impairing dendrite and synapse development; and (2) genes involved in NSC proliferation and neuronal protection (e.g., GALANIN, NG2, NGB), impairing proliferation of aNSCs (Figure 9C) [16], [18], [23], [25]. On the other hand, overexpression of TET3 leads to a decrease in 5hmC levels, disrupting functional generation of mature olfactory sensory neurons from IPCs [18].

MBD1 and MeCP2 readers are other key epigenetic regulators of adult neurogenesis. Studies have found that MBD1 preferentially binds to hypermethylated promoters in the hippocampus and serves to suppress expression of basic fibroblast growth factor 2 (FGF2) promoter, a major mitogen for adult NPCs, thus promoting maturation of new-born neurons [22], [16]. On the other hand, MeCP2 binds to the promoter of brain-derived neurotrophic factor (BDNF), which has been shown to stimulate differentiation, maturation, and survival of new-born neurons in the CNS. Therefore, by suppressing BDNF transcription, MeCP2 inhibits neuronal differentiation and maturation of adult hippocampal NPCs. Deficiencies in MBD1 and MeCP2 lead to impaired adult hippocampal neurogenesis; MBD1-deficient mice show increased proliferation and decreased differentiation of hippocampal NPCs, while MeCP2-deficient mice show delayed neuronal maturation (Figure 10) [13], [27].

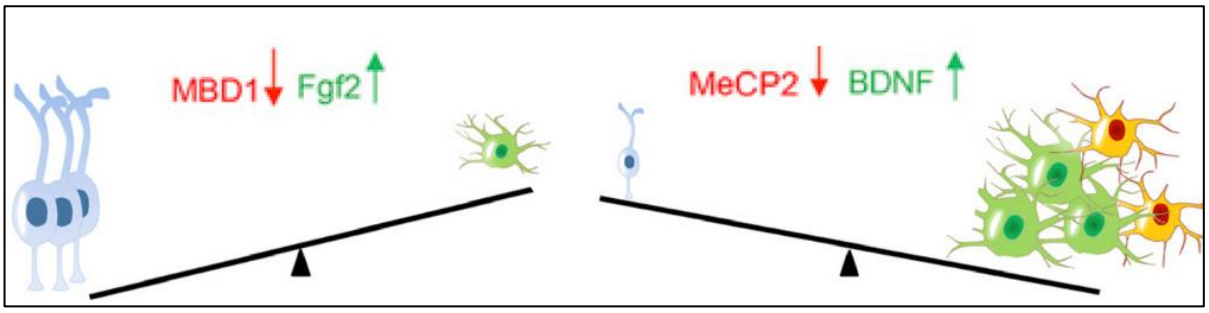

Figure 10. The effects of MBD1 and MeCP2 deficiencies in adult neurogenesis. (Image taken and modified from [22])

3. Histone Modifications

In addition to DNA methylation, histone modifications are another intensively studied epigenetic mechanism that affects gene expression. Efficient DNA packaging in highly ordered chromatin structure limits the accessibility of the DNA template strand for components of the transcriptional machinery. Namely, DNA wraps around a "ball" of eight histone proteins, forming a nucleosome – the single repeating structural unit of chromatin. N-terminal histone tails that stick out from the nucleosome can be reversibly modified by a few dynamic post-translational histone modifications (Figure 1).

These modifications are mediated by a series of enzyme complexes that add/remove various chemical groups onto/from amino acid residues of histone tails, making chromatin more condensed (heterochromatin) or less condensed (euchromatin), hence suppressing or enhancing transcription, respectively. The combination of different histone modifications forms the so-called histone code, which modulates transcriptional expression by directing other regulatory proteins to the chromatin (Figure 11). In addition, crosstalk between histone and other epigenetic modifications coordinates the patterning and maintenance of the transcriptional environment.

Acetylation and methylation are the best studied histone modifications, although there are many more such as phosphorylation, ubiquitination, ADP-ribosylation and glycosylation. In general, acetylation of lysine residues, mediated by histone acetyltransferases (HATs) as writers and histone deacetylases (HDACs) as erasers, neutralizes lysines' positive charges, what weakens the bond between histone core and DNA strands and makes the chromatin more open, thus allowing transcription. On the other hand, histone methylation, mediated by a range of histone methyltransferases (HMTs) as writers and histone demethylases (HDMs) as

erasers, is associated with transcriptional activation or repression, respectively, depending on the specific residue that gets modified.

During neurodevelopment, dynamic epigenetic changes of chromatin accessibility are necessary to allow the transition from a stem cell-like state to more differentiated progeny. Both histone methylation and acetylation are known to have fundamental roles in regulating neurogenesis [16], [28], [29].

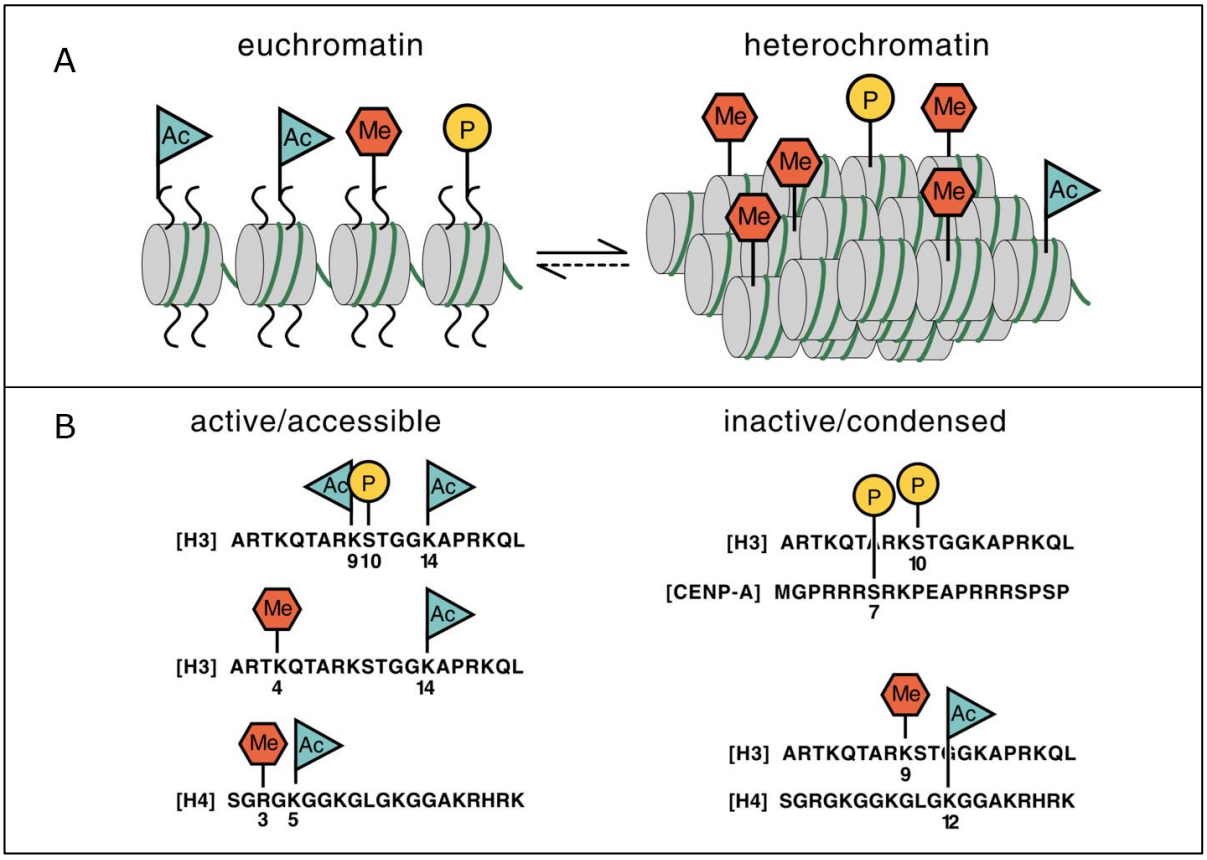

Figure 11. A| Schematic representation of euchromatin and heterochromatin containing acetylated (Ac), phosphorylated (P), and methylated (Me) histone NH2-termini. B| Examples of combinatorial histone modifications that are likely to represent "imprints" for active or inactive chromatin. (Image taken and modified from [30])

3.1. Histone Modifications in Embryonic Neurogenesis

Recent studies have shown that the chromatin of early-stage NPCs is more open (i.e. less condensed) and dynamic than that of late-stage and lineage-committed NPCs, what collides with the fact that neurogenic potential of these cells becomes restricted during the course of brain development. As

the main chromatin modulators, histone modifications play an important role in this switch from neuro- to gliogenesis by indirectly altering expression of numerous genes involved in self-renewal and differentiation stages of NPCs [28].

In terms of histone acetylation, both writers and erasers, HATs and HDACs, play a role in the developing brain. KAT6B is a member of the MYST family HATs, strongly expressed in the VZ, that seems to regulate differentiation of NPCs, since its mutation in mice causes defects in the cortical development [22], [31]. Another HAT, CREB-binding protein (CBP), was shown to directly bind and acetylate H3K9/14 at gene promoters involved in neuronal and glial development, up-regulating their expression. The HAT activity of CBP is known to be triggered by CREB, a generic transcriptional activator that regulates secretion of BDNF. Accordingly, both CREB and CBP are necessary for differentiation of embryonic NPCs [29].

Studies have revealed particularly important functions of two deacetylases, HDAC1 and HDAC2, in regulating differentiation of NPCs in the developing brain. Proliferating NPCs have higher expression of HDAC1 and lower expression of HDAC2. Neuro-differentiation of NPCs is characterized by H3K9 hyperacetylation, down-regulated expression of HDAC1, and upregulated expression of HDAC2. Therefore, throughout the brain, HDAC1 is primarily detected in glial cells, whereas HDAC2 in postmitotic neurons (Figure 12). It has been proposed that HDAC1 is involved in attenuating neuronal gene expression, while HDAC2 is involved in attenuating glial gene expression [32], [33]. Another important HDAC during NPC differentiation is sirtuin 6 (SIRT6), also known as class III HDACs [27], which limits the expression of TETs by inhibiting expression of pluripotent genes, OCT4 and SOX2. SIRT6 depletion caused a neurodevelopmental delay, characterized by upregulation of TETs, hyperhydromethylation and overexpression of neuroectoderm genes [18].

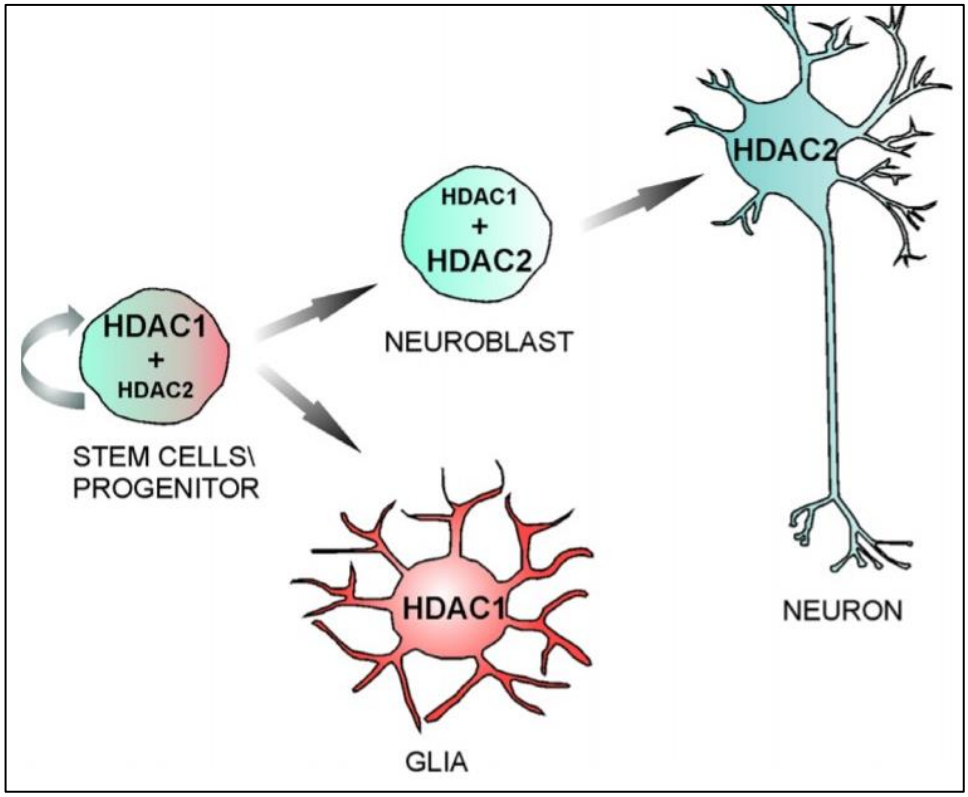

Figure 12. Expression of HDAC 1 and HDAC2 during embryonic neurogenesis. (Image taken from [32])

In addition to histone acetylation, gene regulation by two distinct Polycomb repressive complexes, PRC1 and PRC2, is another well-studied epigenetic mechanism that promotes NPC neurogenic-to-gliogenic transition by repressing neurogenic genes such as NEUROG1. EZH2, a PRC2 component, is responsible for generating repressive H3K27me3, which can be read by PRC1 complex [19], [21], [22]. Deletion of EZH2 before the onset of neurogenesis causes a shift in the balance between NPC self-renewal and differentiation towards differentiation (Figure 13), characterized by an accelerated neurogenic phase with overproduction of BPs and neurons at early stages and their depletion at later stages of development, and an early onset of gliogenesis. Therefore, PRC2 is essential for controlling the rate of neurogenesis [34].

Other key HMTs are BMI1, a PRC1 component that catalyses H3K27 methylation, and Trithorax (TRXG) complex that catalyses H3K4 methylation. BMI1 acts to suppress regulatory regions of the cell cycle

inhibitors p16, p19, and p21, as well as certain neurogenic pathways, allowing the NSC proliferation and delaying the onset of differentiation. Its underexpression causes a depletion of NSCs due to upregulation of mentioned inhibitors. On the other hand, TRXG is a family of transcriptional activators that can dynamically counteract PRC-mediated repression [29], [22]. A member of TRXG family, MLL1, upregulates DLX2 promoter, which encodes a transcription factor required for neuronal differentiation of NSCs residing in the SVZ and OB (Figure 13) [22].

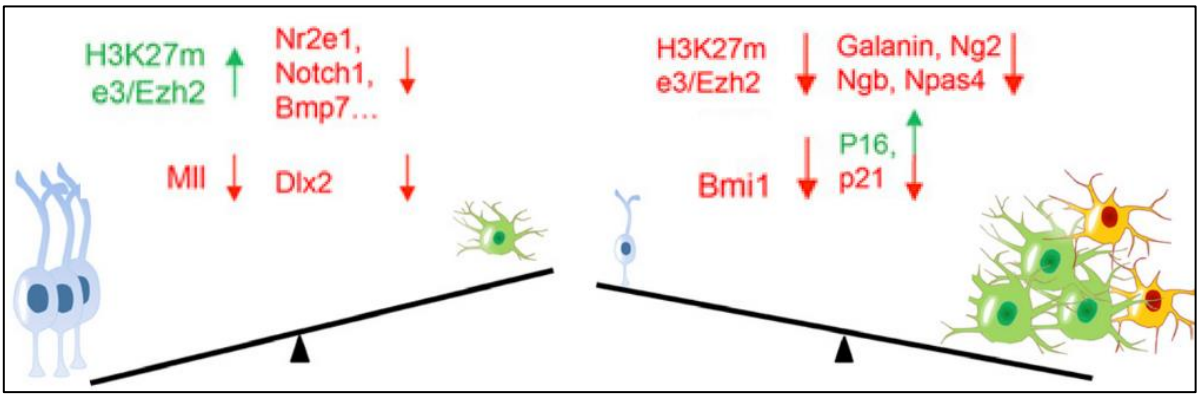

Figure 13. Effects of variable expression levels of certain chromatin modifiers on the balance between self-renewal and differentiation state of NSCs (Image taken and modified from [22])

3.2. Histone Modifications in Adult Neurogenesis

Many writers and erasers of histone modifications have recently been identified to regulate adult neurogenesis. As in the embryonic brain, the writer KAT6B was found to be highly expressed in the adult SVZ, where its deficiency caused reduced proliferation of NPCs and a significantly decreased number of OB interneurons, as well as migrating neuroblasts in the rostral migratory stream. It is proposed that KAT6B-dependent histone acetylation helps to maintain the neurogenic capacity of NPCs in the adult brain [16], [22], [13].

Furthermore, expression of multiple HDACs has been identified and examined in the NSC development. Best studied are HDAC1 and HDAC2, which maintain the self-renewal phase of NSCs in the adult SGZ by silencing

the expression of key neurogenic transcription factors and cell-cycle regulators [35]. However, recent studies suggest that HDAC2 plays a cell-autonomous role during adult, but not embryonic neurogenesis, when it proposedly mediates the silencing of SOX2 expression, a marker of multipotent SGZ NSCs, after neuronal differentiation [13], [35]. Multiple studies on HDACs have shown very conflicting results: from HDAC1/HDAC2 deletion causing severe neurodevelopmental defects; HDAC2 deletion causing abnormal maturation and decreased survival of new-born adult neurons; HDAC3 deletion causing decreased proliferation and differentiation of aNPCs; to HDAC inhibitors (HDACi) inducing neuronal differentiation in embryonic and adult NPCs [13], [27], [29], [35]. Nonetheless, it seems clear that specific HDACs are required for adult neurogenesis, but they seem to have cell-lineage-specific expression patterns and distinct roles in different stages and regions of the brain [27]. More studies are needed to shed light on the exact functions of individual HDACs, as well as their interdependence, in the adult neurogenesis.

As for histone methylation, Polycomb and Trithorax groups of proteins are also implicated in regulating adult neurogenesis. The BMI1 subunit of PRC1 is essential for self-renewal and maintenance of aNPCs, as its lack of expression causes depletion, whereas its overexpression causes augmentation of the number of adult SVZ NPCs [13]. It has been proposed that BMI1-mediated methylation of H3K27 silences differentiation-specific genes, thereby promoting self-renewal as a form of "cellular memory" [35]. Furthermore, a TRXG family member MLL1 has been identified to bind to DLX2 promoter, which encodes an important transcription factor for migration of neuroblasts and development of OB interneurons. It has been recently proposed that MLL1 primarily acts by recruiting JMJD3, a class of H3K27me3 demethylase, to the DLX2 promoter, rather than catalysing H3K4 methylation. Consequent removal of repressive H3K27 methylation marks allows DLX2 transcription that is required for proper neuronal differentiation of SVZ NPCs. Finally, it appears that Polycomb and Trithorax

families cooperate to regulate adult neurogenesis, as NSCs switch from PRC-mediated self-renewal state to TRXG-mediated neuronal differentiation state [13], [27], [29], [35].

Proper interaction of MLL1 and JMJD3 in adult neuronal differentiation is also dependant on the regulation of JMJD3 gene, mediated by the silencing mediator for retinoid or thyroid hormone receptors (SMRT). SMRT represses expression of JMJD3 gene, maintaining the NSC state, while its depletion results in upregulation of JMJD3, which demethylates a number of neurogenic genes, causing neuronal differentiation [22].

As JMJD3 is necessary for TRXG-mediated neuronal differentiation, so is another HDM necessary for PRC-mediated NSC proliferation. H3K4me3 lysine demethylase LSD1 acts as a transcriptional co-repressor of nuclear receptor TLX, which is an essential regulator of aNSC proliferation. TLX recruits LSD1 and other HDACs to repress transcription of cell-cycle genes, such as the cyclin-dependent kinase inhibitor p21 and the tumour suppressor gene PTEN, and thus maintains NSCs in an undifferentiated and self-renewable state [22]. Other examples of HDMs associated with neurodevelopment are UTX H3K27me3 demethylase needed for proper hippocampal function and PHF2 H3K9me3 demethylase that regulates the cell cycle of NPCs during DNA damage and genome instability [19].

In order to generate neurons continuously throughout life, aNSCs depend on several histone writers and erasers, whose specific mechanism of orchestration remains to be elucidated.

4. Non-coding RNAs

Rapid progress of next-generation sequencing technologies revealed a surprising fact that the vast majority of the human genome is transcribed into RNAs that do not translate into proteins [36]. These non-coding RNAs (ncRNAs), generally, have functions in regulating gene expression at the transcriptional and post-transcriptional level. Well-known classes are tRNAs and rRNAs that serve as mediators of translation, however, many additional classes of ncRNAs exist that play critical roles in a wide range of cellular processes from DNA transcription, pre-mRNA splicing, protein synthesis to DNA packaging and gene regulation [13]. The simplest classification is based purely on length, that is number of nucleotides (nts), with classes of ncRNAs divided into two main groups; small ncRNAs (<30 nts) and long ncRNAs (>200 nts) [37], [22].

Small ncRNAs are mainly derived from larger RNA precursor molecules that are cleaved with the help of RNase III-family enzymes (usually DROSHA and DICER) and include microRNAs (miRNAs), short interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), small nucleolar RNAs (snoRNAs), as well as other emerging classes [38]. Their small size and sequence complementarity allow them to participate in epigenetic regulation of gene expression by interacting with other RNAs and protein components [27]. miRNAs (~22 nts) are derived from hairpin-shaped structures present in long ncRNA precursors and are modified in two consecutive cleavage steps by DICER and DROSHA complexes. Bases of mature miRNAs are paired with target mRNAs to inhibit translation or direct degradation of mRNA via RISC complex. siRNAs (~21 nts) are derived from double-stranded RNA precursors that are cleaved by DICER. In a similar fashion to miRNAs, siRNAs reduce mRNA expression levels by degradation or suppressed translation. Finally, piRNAs (28–33 nt long) are formed from single-stranded RNA precursors by poorly characterized DICER pathway and are involved in transposon silencing in germline and somatic cells [38].

Long ncRNAs (lncRNAs) are longer than 200 nts and include the majority of ncRNA transcripts. They originate from various coding or non-coding sequences including intergenic regions, introns, enhancers, promoters, and exons. In contrast to small ncRNAs, they have greater diversity, as well as flexibility in terms of their functions and mechanisms of action. They regulate gene expression at many levels, from recruitment of regulatory proteins and modulation of chromatin at transcriptional level to inhibition of miRNAs by serving as miRNA sponges. One of the reasons for their broad spectrum of actions lies in their ability to engage and complex with various macromolecules [39]. For example, lncRNAs can interact with proteins, modulating their activity or localization within the cell, or they can be processed resulting in different types of small regulatory RNAs. Therefore, one of the main topics of today's studies on lncRNAs are their interactions with chromatin-modifying proteins [38].

In contrast to DNA methylation and histone modifications, precise mechanisms of RNA-based regulation of gene expression are much less known. However, recent studies have shown that ncRNAs are associated with various stages of NSC fate determination during brain development. Prominent research findings on the roles of miRNAs and lncRNAs in regulating embryonic and adult neurogenesis are discussed below.

4.1. The Role of microRNAs in Neurogenesis

Among ncRNAs, miRNAs and their roles in neurogenesis have been the most extensively studied. Namely, a large number of different miRNAs regulate NSC proliferation, differentiation and maturation during neurogenesis [40]. miR-19, miR-17-92 and miR-184 are found to promote the proliferation of NSCs; miR-19 does so by reducing the tumour suppressor gene PTEN expression; miR-17-92 cluster does so by silencing TBR2 expression, which is found to induce IPC fate; and miR-184 does so by reducing the levels of MBD1 and Numb-like (NUMBL) mRNAs. As mentioned in the Chapter 2.2., MBD1 is involved in aNSC fate commitment, but it is also found to regulate the expression of several miRNAs, including

miR-184. By silencing miR-184 expression, MBD1 causes an increase in NUMBL protein, an important regulator of cortical brain development, leading to differentiation and neurogenesis. Therefore, MBD1, miR-184, and NUMBL form a regulatory loop that helps control the balance between aNSC self-renewal and differentiation [16], [18], [40], [7].

By contrast, miR-9, miR-137, miR-124, and let-7b are found to, generally, promote neuronal differentiation. miR-9 does so by repressing the levels of TLX and LSD1, whose roles are described in the Chapter 4.2. Expression of miR-9 is regulated depending on the phase of neurogenesis. During NSC proliferation, TLX recruits transcriptional repressors, such as HDAC5 and LSD1, to the miR-9 locus to inhibit miR-9 expression. These repressors dissociate from the locus at the onset of NSC differentiation, leaving transcriptional activators, such as CREB, to increase miR-9 expression (Figure 14) [40]. One study demonstrated that miR-137 forms a similar regulatory loop with TLX and LSD1 in aNSCs, which, analogous to the regulatory loop of TLX-miR-9-LSD1, mediates the fine balance between NSC self-renewal and differentiation [41].

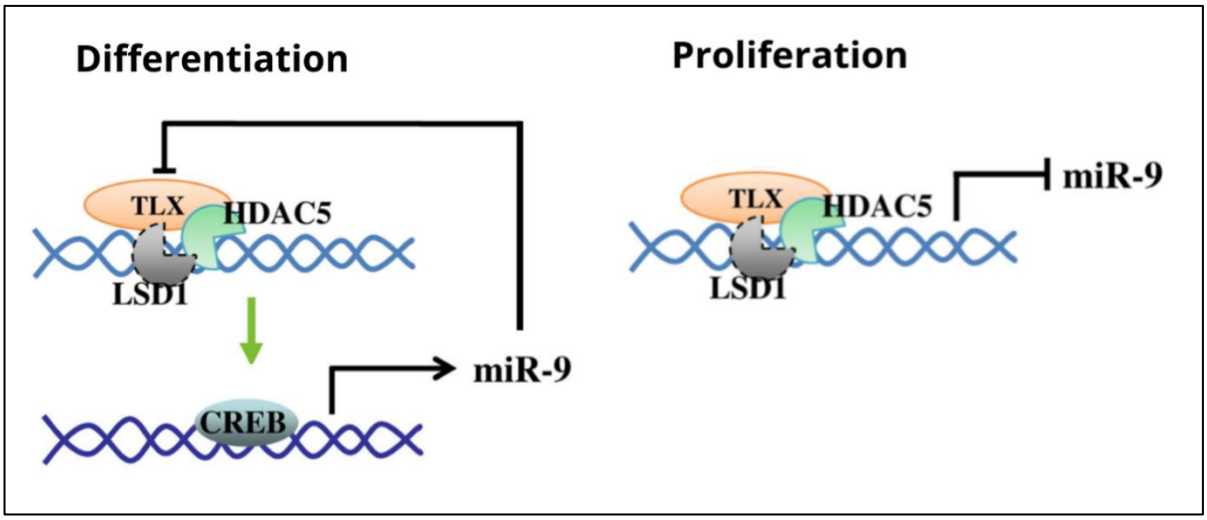

Figure 14. The regulatory loop between miR-9 and their regulators in NSC proliferation and differentiation. (Image taken and modified from [40])

Another study showed that inhibition of MeCP2 led to increased miR-137 and decreased PTEN expression, suggesting that overexpression of miR-137 inhibits PTEN, what, on the contrary, maintains NSCs in a proliferative

state [42]. In favour of this finding, other studies showed that MeCP2-mediated repression of miR-137 upregulates Ezh2 expression [43], which has been shown to recruit DNMT to PTEN promoter destined for DNA methylation and gene repression, maintaining NPCs in a self-renewable state [44]. Specific mechanisms that underlie diverse functions of miR-137 remain to be elucidated. miR-124 mediates neuronal differentiation through five identified mechanisms; by downregulating SCP1, BAF53a, SOX9, PTBP1, and EPHRIN-B1; and inhibits differentiation by targeting pro-neuronal gene NEUROD1. miR-124 expression can be repressed by the binding of the element-1 silencing transcription factor (REST) (Figure 15) [40].

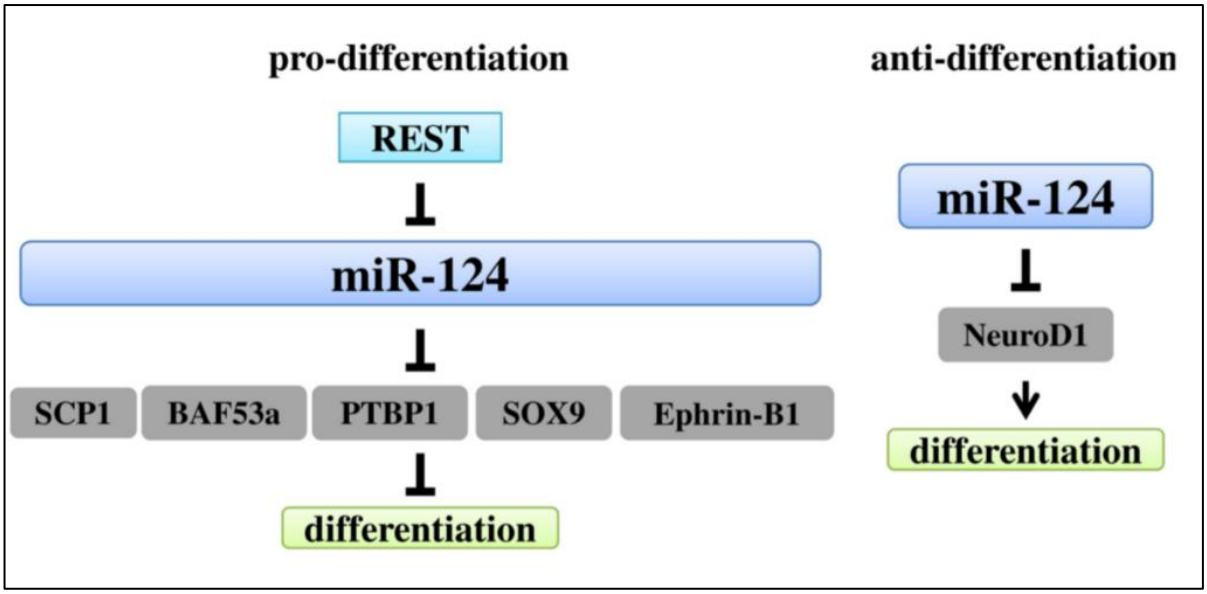

Figure 15. Regulation and functions of miR-124. (Image taken and modified from [40])

Another regulatory loop that controls neural differentiation is the interplay between let-7 family of miRNAs and LIN-28. LIN-28 is a pluripotent factor expressed in ESCs that inhibits maturation of let-7 miRNAs during NSC proliferation. Upon neuronal and astroglial differentiation, multiple let-7 miRNAs suppress expression of LIN-28, certain cell cycle regulators and proneural genes, and thus inhibit further proliferation. In addition, let7b family member has been shown to target TLX and the cell cycle regulator CCND1, which encodes cyclin D1 [7]. Several miRNAs, including miR-184,

let-7b, miR-137, miR-9 and miR-124, are found to have significant regulatory roles in adult hippocampal neurogenesis [16], [27].

4.2. The Role of Long Non-coding RNAs in Neurogenesis

Recent studies have identified dynamic expression profiles of numerous lncRNAs in embryonic and adult brain, however, their functions in neurodevelopmental processes are much less understood. It seems that distinct lncRNA types are involved in different stages of neurogenesis and, like miRNAs, they can regulate diverse downstream targets including coding genes, transcriptional factors and small ncRNAs [45], [46]. EVF2 (or DLX6OS1, from DLX6 opposite strand transcript 1) was the first known functional lncRNA to be involved in neurodevelopment. EVF2 regulates the generation of GABAergic interneurons in the SVZ of the developing forebrain by recruiting transcriptional activators DLX and MeCP2 to the adjacent DLX5 and DLX6 intergenic regions, enhancing their expression and neuronal differentiation (Figure 16). Knockout of EVF2 results in reduced numbers of GABAergic interneurons within the early postnatal hippocampus and DG, what leads to defects in synaptic inhibition throughout life [46].

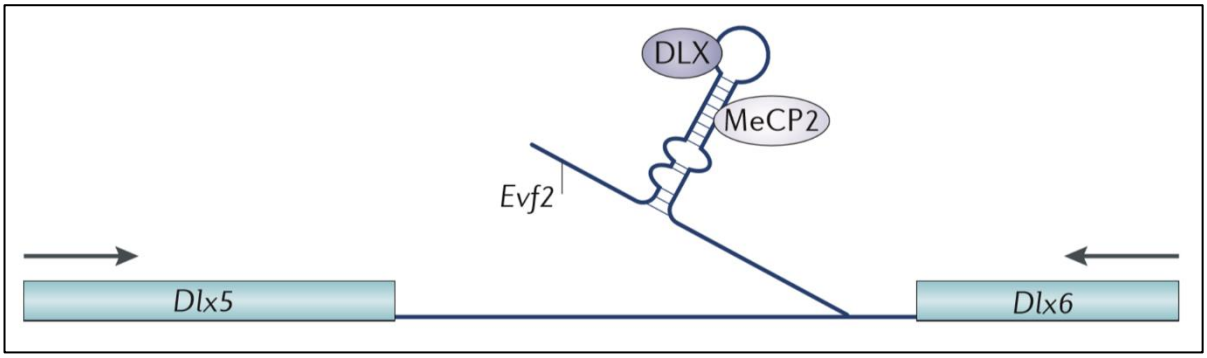

Figure 16. Function of lncRNA EVF2. (Image taken and modified from [16])

From the previous example it may be observed that lncRNAs may control neurodevelopment through influencing expression of proximal protein-coding genes. More examples include SOX1OT (SOX1 overlapping transcript) and SOX2OT (SOX2 overlapping transcript) that are transcribed from SOX1/SOX2 and mediate their suppression, thus leading to neuronal

differentiation [46], [47]. The lncRNA RMST (rhabdomyosarcoma 2-associated transcript) has been identified to facilitate the binding of SOX2 to proneural target genes, such as ASCL and NEUROG1, thus promoting neuronal fate commitment in the midbrain [27], [7]. In contrast to lncRNAs mentioned so far, some neuronal lncRNAs are involved in blocking neuronal differentiation. For example, lncRNA PNKY interacts with the splicing regulator PTBP1 and regulates the expression and alternative splicing of a common set of transcripts in order to "restrain" neuronal commitment in embryonic and postnatal NSCs [19], [48].

Several lncRNAs are involved in neuronal-glial fate specification and oligodendrocyte lineage maturation. LncRNA UCA1 (human urothelial carcinoma associated 1) enhances expression of miR-1 and decreases expression of its target gene, HES1, strengthening neuronal fate commitment. Additionally, lncRNA-lnc-OPC contributes to glial differentiation and oligodendrogenesis [47]. lncRNA MIAT controls proliferation versus differentiation by regulating splicing of cell fate determinants, such as WNT7B [7]. Recently discovered lncRNA linc-ROR has been shown to work as miRNA sponge, regulating all three essential ESC renewal transcription factors OCT4, NANOG and SOX2 through a feedback loop [45]. lncRNA DLX1-AS is selectively required for the SVZ neuronal differentiation, whereas SIX3-OS plays a role in both neuronal and oligodendrocyte differentiation [45]. Depletion of these two lncRNAs in DG and SVZ NPCs leads to fewer new-born neurons and increased astrocyte differentiation in aNSCs [16], [27].

Further research is needed to highlight the roles of individual lncRNAs, their interconnection, cooperation with other epigenetic modulators, and their underlying mechanisms of action in regulating adult and embryonic neurogenesis.

5. Epigenetic Crosstalk

As previously shown, a long list of epigenetic mechanisms, including DNA methylation, histone modification, and ncRNAs, contribute to different aspects of embryonic and adult neurogenesis. In fact, these mechanisms are highly intertwined and rely on each other, as well as other cellular signalling pathways, for generating diverse and specialized populations of neurons. A few examples of how crosstalk among epigenetic pathways (Figure 17) control the neurogenic process are shown and described below.

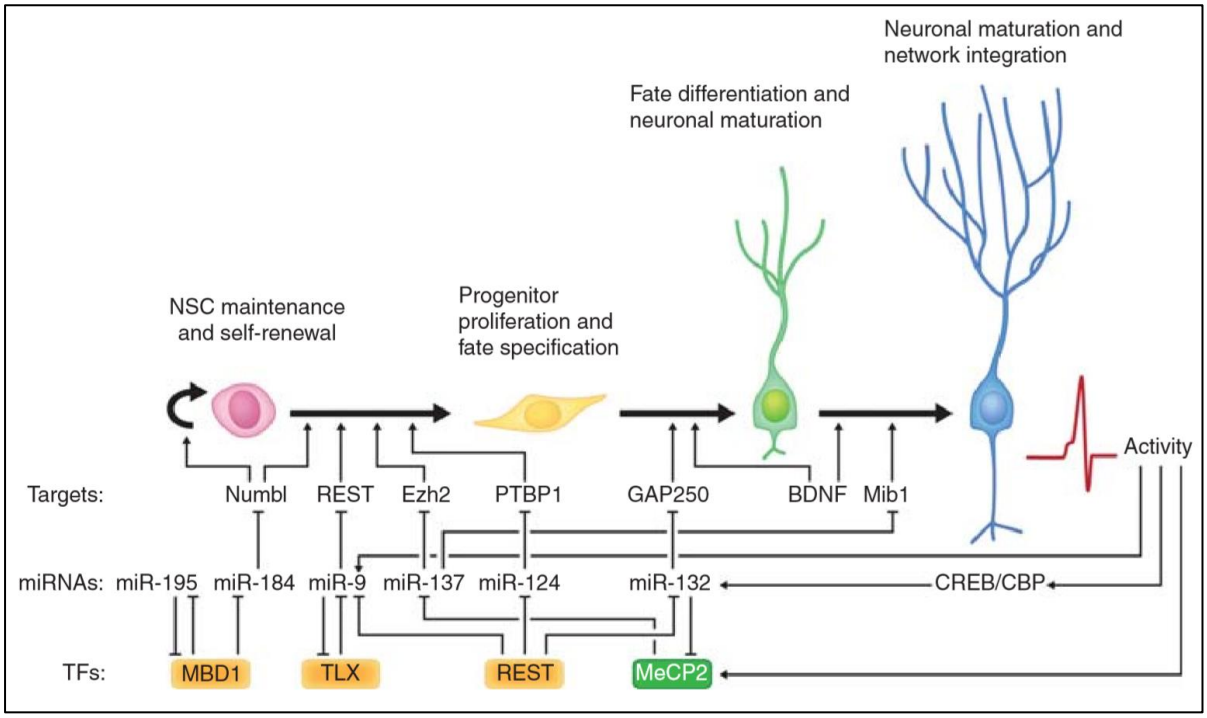

Figure 17. Crosstalk between key epigenetic and transcriptional mediators in regulating neurogenesis. (Image taken from [27])

5.1. The Roles of REST

REST is a transcriptional repressor that regulates neuronal development by orchestrating a complex network of transcriptional and epigenetic events. It mediates transcriptional repression of neuron-specific genes in non-neuronal cells by recruiting cofactors, mSin3/HDAC complex and CoREST complex, which then recruit a massive array of epigenetic modifiers, including HDACs, DNMTs, MECP2, and ncRNAs, to the REST binding site

(RE1) site within neuronal genes. For example, MeCP2 binds to methylated CpGs and recruits HDACs, thus maintaining neuronal genes in a heterochromatin state. Moreover, REST can repress expression of certain miRNAs involved in neuronal differentiation, including miR-9, miR-124, and miR-132, as well as expression of lncRNA RMST. The relationship goes both ways with REST being a repressor as well as a target of several miRNAs that it regulates (Figure 17) [25], [49], [50]. For instance, the regulatory loop with miR-9 is explained in the Chapter 4.1. Therefore, REST maintains the NSC pool and prevents precocious neuronal differentiation by linking complex epigenetic and transcriptional mechanisms.

5.2. The Roles of MeCP2 and MBD1

Another example of crosstalk is the cooperation between readers of DNA methylation, MeCP2 and MBD1, and other epigenetic factors during NSC fate commitment. As mentioned in the Chapter 2.2., MeCP2 represses the expression of developmental genes (e.g., BDNF) directly by binding to the gene promoter regions or indirectly through miRNA pathways. MeCP2 is a target of miR-132 and a repressor of miR-137. miR-132 reduces MeCP2 levels upon neonatal neuronal differentiation and maturation, whereas MeCP2 and SOX2 reduce miR-137 levels during aNSC self-renewal. Therefore, MeCP2 is regulated by neuronal activity and in part through CREB-regulated miR-132. On the other hand, MBD1 promotes NSC differentiation through either direct regulation of critical neurogenic genes (e.g., FGF2) or indirect crosstalk with miRNA pathways (Figure 17) [49]. Crosstalk with miR-184 is discussed in the Chapter 4.1.

To conclude, interactions between different epigenetic and transcriptional enzymatic complexes play key roles in neuronal differentiation. A tentative summary of epigenetic crosstalk in embryonic and adult neurogenesis, respectively, is displayed in Figure 18. Nonetheless, additional research in this field is needed for better understanding of the intertwined epigenetic mechanisms of action in regulating neurogenesis.

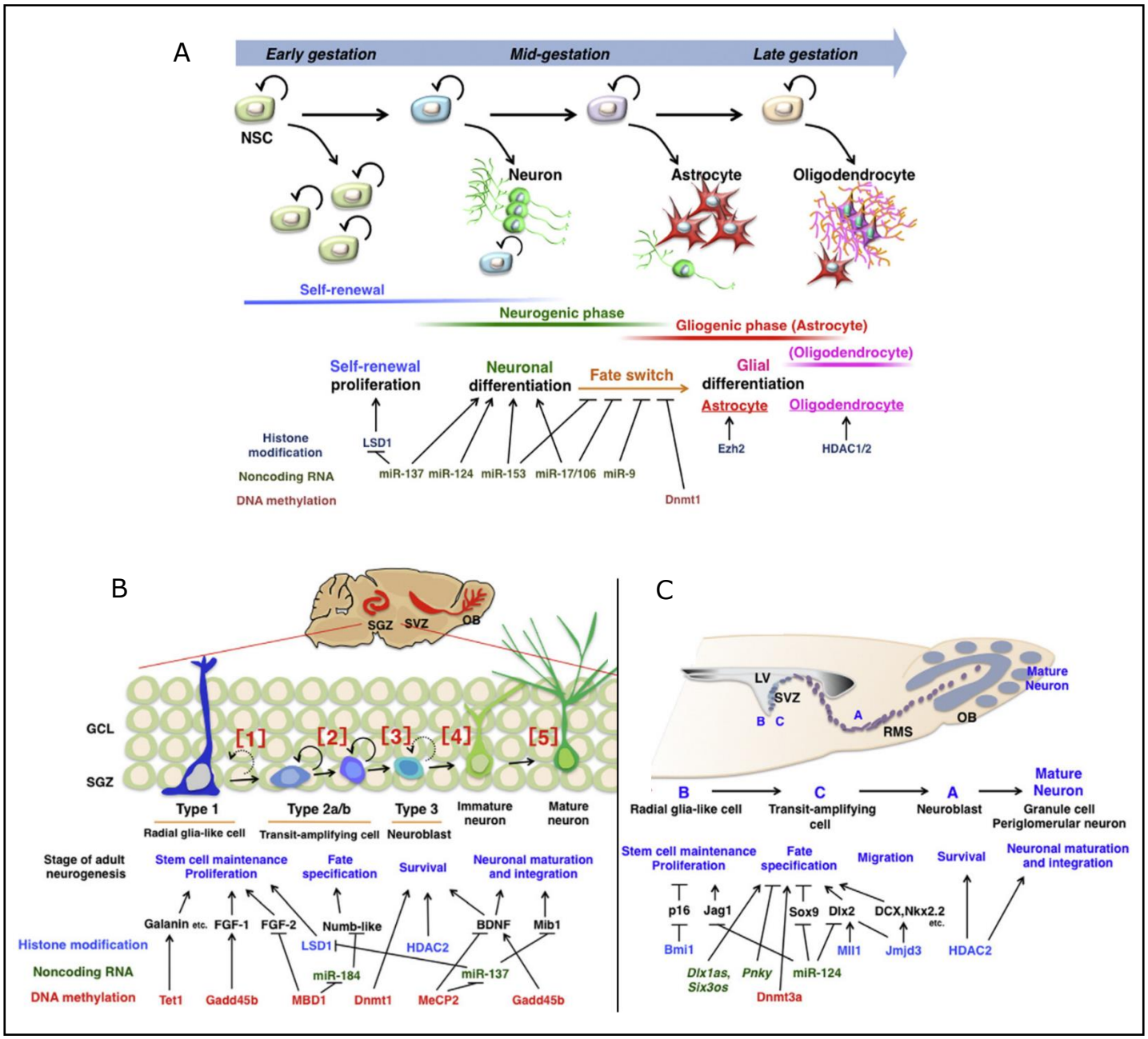

Figure 18. Crosstalk between key epigenetic mechanisms A| during embryonic neurogenesis, B| during adult hippocampal neurogenesis C| during adult SVZ and OB neurogenesis (Image taken and modified from [25])

6. Conclusion

Neurogenesis is a spatially and temporally orchestrated process that requires an intricate network of epigenetic mechanisms, including DNA methylation, histone modifications, and ncRNAs, to generate diverse and functionally distinct neuronal populations in both embryonic and adult CNS. Many examples discussed in this review suggest that epigenetic mechanisms are involved in all aspects of neurogenesis, from maintenance of NSC multipotency to neuronal differentiation, survival, and maturation. In addition, an extensive crosstalk among multiple epigenetic and transcriptional pathways ensures that the correct set of genes is expressed in distinct neurogenic stages, and misregulation of these processes may lead to neurodevelopmental deficits and neurological diseases. With emerging new technologies, such as high-throughput sequencing and genomic editing, our understanding of the roles of epigenetic modifications in neurogenesis, as well as underlying molecular mechanisms, is only beginning to unravel and enormous progress is being made.

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8. Curriculum Vitae

PERSONAL INFORMATION

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Skype katarina.vagaja

Date of birth 10/03/1999 | Nationality Croatian

EDUCATION

09/2020– **Neurosciences (research), Master's Degree**
Vrije Universiteit Amsterdam (Netherlands)

10/2017–Present **Biotechnology and Drug Research, Bachelor Degree**
Department of Biotechnology, University of Rijeka (Croatia)

09/2013–07/2017 **High School Diploma**
Gimnazija Andrije Mohorovičića Rijeka (Croatia)

ADDITIONAL EDUCATION

02/03/20–24/06/20 **Marketing, Faculty of Economics and Business**
University of Rijeka (Croatia)

Gained strong skills in marketing data analysis using Excel pivot tables. My market research project achieved the highest score (97%), making it best in class. Grade: A (95%), 6 ECTS

06/07/19–03/08/19 **VU Amsterdam Summer School**
Vrije Universiteit Amsterdam (Netherlands)

Neurodegenerative Diseases: From Lab to Patient and Back (2 week course)

Covered clinical aspects (etiology, pathology, genetics, diagnostics and treatment) and methodology (cell and animal models, microscopy, physiology and behaviour) to study brain diseases with focus on Alzheimer's disease

Single-Cell Technologies in Life Sciences (2 week course)

Covered principles of fluorescence cytometry, imaging flow cytometry, mass cytometry, essential analysis data methods for multicolor cytometry; principles of light microscopy, electron microscopy, light sheet microscopy, and essential data analysis with Image J

09/2012–05/2017 **The Rijeka Youth Theatre**
English-language drama school, https://trytheatre.org/ (Croatia)

09/2010–06/2015 **Elementary Music School Diploma**
Music School Mirković Opatija (Croatia)

Courses: Piano, Solfeggio

WORK EXPERIENCE

11/2019 **Student Laboratory Assistant on the Course "Cellular and Molecular Biology"**
Department of Biotechnology, University of Rijeka (Croatia)

04/2019 **Student Laboratory Assistant on the Course "General Chemistry"**
Department of Biotechnology, University of Rijeka (Croatia)

11/2018–03/2019 **Promotion Demonstrator of Dietary Supplements**
Sensapharm d.o.o., Zagreb (Croatia)

07/2016–Present **Tourist Information Clerk**
Kvarner County Tourist Board, Opatija (Croatia)

VOLUNTEER EXPERIENCE

08/2019 **Volunteer in the Laboratory for Molecular Neurobiology**
Department of Biotechnology, University of Rijeka, http://neuroreg.uniri.hr/

Biotechnology Student Association at University of Rijeka (USBRI)
E-mail: usbri@uniri.hr; http://www.usbri.uniri.hr/

10/2018–06/2020 **Vice President**

Responsibilities: volunteer and project coordination; organization of meetings, assemblies, workshops and events; fundraising; social media and marketing management; occasional photographer; representing the Association on radio shows, Student Day Festival, Camp for Freshmen, and other manifestations

Project Manager

12/2019 Co-organizer of the 6th conference "Future and Perspective of Biotechnology" that aims to present STEM students career and hiring opportunities in the industrial and academic sector

04/2019 Organizer of the manifestation "Open Sports Day" for students of University of Rijeka with aim to promote healthy lifestyle, sports and diet and to improve students' health

Volunteer

04/2018–12/2019 In the project "Travelling Scientists" – popularization of science by performing "kitchen chemistry" experiments and teaching children the basics of physics and chemistry

2018/19; 2019/20 In the project "Student Mentors" – mentoring two 1st year students, helping them with orientation at the Department of Biotechnology

04/2018; 04/2019 On Open Day of Biotechnology – isolation of banana's DNA, performing kitchen chemistry for youngsters

03/2019 In the project "Campus Carnival Group" – helping with organization and costume making

09/2018 In the project "Summer School of Chemistry"– introducing 1st year students to the basics of laboratory work

09/2018 At the European Researchers' Night – helping with organization, promoting the manifestation at Rijeka's elementary schools

Association "Centre for the Culture of Dialogue" (CeKaDe)
E-mail: cekaderi@gmail.com; Rijeka (Croatia)

10/2018–06/2019 **Mathematics Tutor**

Teaching Mathematics to elementary school children through "My Place under the Sun" – an inclusion program for children with socioeconomic disadvantages

TRAINING

02/2020 **Two-day workshop: "Fundamentals of Scientific Writing"**
Tutor Filip Đerke, dr.med.

10/09/19–18/09/19 **Erasmus+ Training Course "Edu Boards: Exploring Educational Board Games"**
MTÜ Shokkin Group (Estonia)

Covered theory of game-based learning methods and gained tools for developing educational board games used in youth work. In a team of four, developed an educational card game "You got scammed!" on the topic of media literacy and online data protection. Link for the game: http://shokkin.org/wp-content/uploads/2017/05/DataSecurity_Eduboards-1.pdf

03/12/19 **Workshop "Pitching Training"**
Realizator 2019, University of Rijeka Foundation

12/11/19 **Workshop "Tips and Tricks for Preparing and Writing Business Solutions"**
Realizator 2019, University of Rijeka Foundation

07/11/19 **Workshop "Generating Business Ideas: Techniques & Tools"**
Realizator 2019, University of Rijeka Foundation

20/12/18 **Workshop "From Idea to a Project"**
Student Council of the University of Rijeka (SZSUR)

Acquired fundamental knowledge and skills in writing project proposals: formulation of problems, users, goals, activities, expected results and budget planning

CERTIFICATIONS

YouthPass: Mobility of Youth Workers

Europe-wide validation system for non-formal learning within Erasmus+: Youth in Action Programme; credential ID HVP8-5PCS-D5EN-V3CP; issued Sep 2019 by Shokkin Group

CONFERENCES

28/05/20 **Mind Over Matter (online symposium), passive participant**

Study Association Flow at Tilburg University, Netherlands

05/12/19 **6th Conference "Future and Perspective of Biotechnology", active participant**

Biotechnology Student Association at University of Rijeka, Croatia

Active participant – **panel moderator** – in discussion with Biotechnology alumni

25/09/19–28/09/19 **HDBMB Congress "Crossroads in Life Sciences", passive participant**

Croatian Society of Biochemistry and Molecular Biology, Lovran, Croatia

17/05/19–19/05/19 **2nd Congress of Biotechnology Students, passive participant**

Student Association PROBION at University of Zagreb, Croatia

12/04/19 **Inspire Me Conference Rijeka, passive participant**

17/12/18 **5th Conference "Future and Perspective of Biotechnology", passive participant**

Biotechnology Student Association at University of Rijeka, Croatia

08/11/18–10/11/18 **International Biomedical Student Congress, passive participant**

Student Association FOSS at the Medical Faculty of Rijeka, Croatia

HONOURS AND AWARDS

06/2020 **3rd place on Lumen Business 2020 – National Case Study Competition**

For solving the business case "Creating an integrated marketing strategy for La Roche-Posay acne campaign" by L'Oreal Adria d.o.o.

12/2019 **1st place on Realizator 2019 – Regional Case Study Competition**

For solving the business case "How to Prevent Vandalism in the City of Kastav"

11/2014–12/2020 **The City of Opatija Scholarship for Academic Excellence**

MEMBERSHIPS

06/2019-06/2020 Croatian Society of Biochemistry and Molecular Biology (HDBMB)

04/2018-06/2020 Biotechnology Student Association at University of Rijeka (USBRI)

PERSONAL SKILLS

Mother tongue: Croatian

Foreign languages

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
	TOEFL Test 86% (taken December 2016)				
English	C2	C2	C2	C2	C2
	Deutsches Sprachdiplom (DSD) A2				
German	A2	B1	A2	A2	B1
	3 year course at Alliance Française Rijeka				
French	A1	A1	A1	A1	A1

Levels: A1 and A2: Basic user - B1 and B2: Independent user - C1 and C2: Proficient user
Common European Framework of Reference for Languages

Communication skills	▪ Excellent public speaking skills gained from acting in English language drama school, my duties as the vice president of USBRI, my role as a panel moderator on USBRI Conference, and pitching on case study competitions
	▪ Effective written and verbal communication skills gained from University assignments, writing and implementing USBRI project proposals, participation on Realizator and Lumen Business, and experience as a tourist information clerk
Organisational / managerial skills	▪ Skilled in non-profit organisation and project management, public speaking, leadership
	▪ Goal-oriented and results-driven
Digital skills	▪ Competent with Microsoft Office programmes (Word, Excel, PowerPoint)
	▪ Avogadro, GAMESS, Chimera, MacMolPlt, KinTek, PyMol, VMD
	▪ Good graphic design skills using Canva
Other	▪ Passionate about epigenetics and its role in all aspects of life
	▪ Favourite book “The Biology of Belief” by Bruce H. Lipton, PhD