



# The genetic and epigenetic journey of embryonic stem cells into mature neural cells

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Epigenetic changes occur throughout life from embryonic development into adulthood. This results in the timely expression of developmentally important genes, determining the morphology and identity of different cell types and tissues within the body. Epigenetics regulate gene expression and cellular morphology through multiple mechanisms without alteration in the underlying DNA sequences. Different epigenetic mechanisms include chromatin condensation, post-translational modification of histone proteins, DNA cytosine marks, and the activity of non-coding RNA molecules. Epigenetics play key roles in development, stem cell differentiation, and have high impact in human disease. In this review, we will discuss our current knowledge about these epigenetic mechanisms, with a focus on histone and DNA marks. We will then talk about the genetics and epigenetics of embryonic stem cell self-renewal and differentiation into neural stem cells, and further into specific neuronal cell types.

**Keywords: epigenetics, embryonic stem cells, neural stem cells, neurogenesis, gliogenesis, homeobox transcription factors, regenerative medicine**

## INTRODUCTION

Life begins upon fertilization of a haploid egg and a haploid sperm. The resulting zygote will subsequently undergo many cellular divisions in a tightly regulated manner, as part of a complex developmental journey. The underlying genetic code remains the same in all cells within an individual, yet an enormously diverse population of cell types will be generated, which will constitute a multicellular organism. By controlling gene expression, epigenetic mechanisms instruct cellular identity and morphology, greatly contributing to the vast assortment of cellular phenotypes (Barros and Offenbacher, 2009; Delcuve et al., 2009; Rastegar et al., 2011). The term “epigenetics” was used for the first time by Waddington (1942) as a way of “genotype” controlling “phenotype” during development. Since then, we have come a long way and the field of epigenetics has been revolutionized substantially. The word “epigenetics” is named after the Greek word, “epi” that means “on,” or “above” the “genetics.” Epigenetics are known as inheritable programming in gene expression that control cellular morphology and identity, without changing the corresponding DNA sequences (Delcuve et al., 2009; Rastegar et al., 2011). Accumulation of all epigenetic marks define the epigenome of any given cell, similarly in that all genetic information pertains to the genome, and protein components to the proteome.

Epigenetics are profoundly involved in mammalian development (Barber and Rastegar, 2010), human disease (Choo, 2011; Zachariah and Rastegar, 2012) and embryonic stem cell (ESC) differentiation (Delcuve et al., 2009; Rastegar et al., 2011). For the first time in 1981, ESCs were isolated and expanded *in vitro* from the inner cell mass (ICM) of the developing mouse blastocysts

(Martin, 1981). Mouse and human ESC are pluripotent stem cells that are capable of indefinite self-renewal and differentiation into all three germ layers (Martin, 1981; Pan and Thomson, 2007). The discovery of these properties presented ESC as a perfect tool for investigating early development and modeling human disease. In addition, ESC self-renewal and differentiation properties provide a great promise as an inexhaustible resource for cell-based therapies in human degenerative diseases. These unique characteristics have brought ESCs into the forefront of today’s research. ESC differentiation into somatic nerve cells involves a cascade of molecular events, and signaling pathways from the extracellular microenvironment, to the transcriptional activities within the nucleus. In many neurological disorders, we need to direct ESCs toward specific type of neurons to be suitable for transplantation strategies and in regenerative medicine. Such comprehensive knowledge of gene expression profiles and cell fate commitment of stem cells are essential for future stem cell-based therapy applications and for proper *in vitro* modeling of neurological disorders. This information are particularly essential for most applications in cell-based therapy strategies and in regenerative medical approaches, where pure populations of differentiated cell types are required for successful transplantation. Although our scientific community has made great advancement toward directing ESC to differentiate into particular cell types and lineages, even with our best available protocols, we still end up generating heterogeneous populations of differentiated cells. The challenge of our today’s research is therefore to understand how ESCs execute gene expression profiles to undergo such extensive genomic, proteomic, and epigenetic changes during differentiation. Eventually, we would be able to

benefit from this knowledge to control the cell fate commitment of ESCs toward any desired cell type.

In this review, we will first describe epigenetic programming, with a focus on chromatin structure, histone post-translational modifications (PTM), and DNA marks. We will then discuss ESC self-renewal and differentiation followed by the current state of knowledge about the underlying genetics and epigenetic mechanisms in neural development.

## EPIGENETIC MECHANISMS

### CHROMATIN STRUCTURE

The epigenetic information is mainly carried within the “chromatin” structure, referred to the combination of DNA and DNA-bound histones (Delcuve et al., 2009; Rastegar et al., 2011). The repetitive fundamental unit of the chromatin is termed the “nucleosome,” which is a combination of 146 bp of DNA molecules wrapped around a histone octamer (Kornberg, 1974). The histone octamer is composed of four nucleosomal histones, including two of each histone H3 and histone H4 (one tetramer of H3–2H4) and two dimers of histones H2A–H2B. Nucleosomes are further joined by the linker DNA that is bound to the fifth histone, namely the linker histone H1, and can be visualized as “beads-on-a-string” structure (Olins and Olins, 1974). At higher level of organization, nucleosomes are assembled into a fiber-like arrangement, with a diameter of 10 or 30 nm, depending on the compaction state of the chromatin (Fussner et al., 2011a). However, recent evidence support the idea that the 30-nm fiber does not exist *in vivo* (Lieberman-Aiden et al., 2009), and in case of higher order chromatin structure what actually exists is the densely compacted 10 nm fiber. A chromosome is ultimately comprised of these “fibers” and their associated proteins, which allow the chromatin structure to condense, as can be seen throughout cellular division. The compaction state of chromatin can be segregated into tightly packed, “heterochromatin” and lightly packed “euchromatic” regions; with the latter being accessible to gene transcription pertaining to the expressed genome (Horvath et al., 2001; Ellis et al., 2007; Bassett et al., 2009). As a result, the division of chromatin into euchromatin (actively transcribed genes) is marked by histone acetylation, and the heterochromatin (inactive non-transcribed genes) by particular histone methylation with or without DNA methylation. Different heterochromatin regions are characterized by specific epigenetic marks on the inactive repressed genes (referred to as the constitutive heterochromatin) and inactive silenced genes (referred to as the facultative heterochromatin). The euchromatin and heterochromatin compartments are usually dynamic and the chromatin structure can transition between “opened” and “closed” states by carrying a variety of chemical groups to histone proteins or directly to the cytosine nucleotides; in response to extrinsic factors (Delcuve et al., 2009; Rastegar et al., 2011). In general, epigenetic marks are inheritable and can be passed on through cellular division from the mother cells into the daughter cells, but they are also dynamic and reversible in response to stimuli and signaling pathways. Recently, there is an emerging focus on the molecular mechanisms of extracellular signals translating into epigenetic changes, bringing excitement, and introducing new concepts. This is particularly important toward the future application of epigenetics in novel therapeutic strategies

for diseases that currently have no treatment and epigenetic disorders that are caused by environmental insults. In addition to modification in chromatin structure, histone variants, histone PTM, and non-coding RNAs (ncRNA) can further decrease or inhibit gene expression, adding to the diversity of features, which constitute the epigenome (Delcuve et al., 2009; Malecova and Morris, 2010; Rastegar et al., 2011; Faust et al., 2012).

### HISTONE VARIANTS AND POST-TRANSLATIONAL MODIFICATIONS

Each nucleosome constitutes of 146 bp of DNA wrapped around a histone octamer, with an extra 20–50 bp stretch of DNA called the linker region, which associates with the linker histone H1 (Delcuve et al., 2009). Besides histone H4, histone variants are identified for all other histones and they can influence nuclear chromatin both structurally and functionally (Talbert and Henikoff, 2010). These histone variants are expressed either at the time of DNA replication in S phase (H3.1 and H2A.1) or throughout the cell cycle (H2AZ and H3.3; Mizuguchi et al., 2004; Boulard et al., 2007; Elsaesser et al., 2010; Goldberg et al., 2010; Szenker et al., 2011). In addition to the existence of histone variants, the epigenetic information is further transferred through post-translational modification of histone proteins. Histone PTM include: acetylation, phosphorylation, methylation, ubiquitination, sumoylation, and ADP-ribosylation (Kouzarides, 2007; Delcuve et al., 2009). In general, histone PTM take place on the N-terminal tail of histones, yet in limited cases, they have been found within the core sequence of histones (Delcuve et al., 2009; Barber and Rastegar, 2010). Such examples are the H3K56 acetylation (Tjeertes et al., 2009) and H3Y41 phosphorylation (Dawson et al., 2009), which can occur within the core of histone H3. The existence of the particular covalently bound small chemical molecules on histones or DNA molecules, will determine the epigenetic code at any given locus. Overall, the epigenetic proteins include “writer” molecules, which will deposit these covalent marks; the “erasers” that will remove these marks; and “readers” that interpret these marks and will intermediate or facilitate the intended function. The cumulative action of these three types of epigenetic proteins will eventually determine the gene accessibility state for transcription.

Depending on the specific amino acid residues that histone PTM are attached to, the outcome may be a tighter association of histone proteins with DNA and repression of transcriptional activities; or looser association, allowing transcriptional accessibility of RNA polymerase and affiliated transcriptional machinery (Jenuwein and Allis, 2001; Delcuve et al., 2009; Rastegar et al., 2011). Additions of acetyl groups to the lysines 9 (K9) and 14 (K14) of histone H3 or lysine 16 of histone H4 (H4K16) are performed by K-histone acetyl transferases (KAT), and correspond with open chromatin state (Roth et al., 2001). The lysine residues have a positive charge that leads to their tight association with the negatively charged DNA. The addition of an acetyl group to lysines will eliminate this positive charge leading to a more relaxed association with DNA molecules and as a result the corresponding genomic loci will become more accessible for transcription. Removal of acetylation marks on histones is accomplished by histone de-acetylases (HDAC; Zhang et al., 2002; de Ruijter et al., 2003; Brandl et al., 2009; Delcuve et al., 2009; Rastegar et al., 2011). Phosphorylation of histones usually occurs by addition

of a phosphate group onto a hydroxyl group on serine (S) or threonine (T) causing a negative charge, leading to general relaxation and chromatin de-condensation. A well-known example is histone H3S10 phosphorylation that is associated with transcriptional initiation in many genes (Delcuve et al., 2009). Histone methylation is catalyzed by the activity of lysine methyl transferases (KMT), characterized by the existence of a SET domain. Histone methylation can occur at the lysine (K) and arginine (R) residues of histones, and depending to the degree of methylation and the amino acid residues that become methylated, the outcome would be different. For example, H3K4me3 is associated with active genes, H3K9me3 is a hallmark of constitutive heterochromatin and H3K27me3 marks facultative heterochromatin. Interestingly, H3K9me2 is not associated with constitutive heterochromatin, but rather it marks the silenced genes within the facultative compartment of the heterochromatin. Using specific antibodies raised against each of these particular histone marks, chromatin immunoprecipitation (ChIP) techniques can be used to identify the association of particular genomic loci with each of these epigenetic marks. There are some histone lysine demethylation (KDM) enzymes that can erase the histone methyl marks from active or repressed genes, which would also depend on the amino acid residues that are methylated.

In addition to these histone marks, other modifications are found at the lysine residues such as ubiquitination, which has been found on the histones H2A (K119) and H2B (K120; Delcuve et al., 2009; Rastegar et al., 2011). The molecular mechanism of histone ubiquitination is still not fully understood, but it is likely that it functions in keeping chromatin at the open/active state due to its large molecular size (Calvanese and Fraga, 2012). Another identified histone PTM is the sumoylation that occurs in the core region of the four nucleosomal histones, and it happens at the exact same lysines that can become acetylated or ubiquitinated, in an exclusive manner. Other histone PTM include proline isomerization and ADP-ribosylation, both of which are involved in transcriptional gene regulation. It should be noted that the existence of histone marks on the same amino acids are exclusive and they may not occur at an individual residue, simultaneously. This is one of several forms of histone crosstalks, and here the existence of one histone PTM would prevent the deposition of another form to happen at the same residue. Histone crosstalks are not limited to the same histone molecules; rather, modification of an amino acid on one histone can also influence deposition of a different form of PTM on another histone molecule. In addition to the histone PTM crosstalks, these covalent modifications can direct gene expression through crosstalks with chemical groups on DNA molecules such as DNA methylation or 5-hydroxymethylation (Delcuve et al., 2009; Calvanese and Fraga, 2012).

### DNA MARKS

In addition to methylation of lysine residues within the histone proteins, methylation can also proceed directly on DNA molecules. DNA methylation occurs on the 5' carbon of the cytosine nucleotides (5mC), next to guanine (CpG dinucleotides). DNA methylation is a hallmark of transcriptionally inactive genes and usually correlates with gene repression and silencing (Suzuki and Bird, 2008; Delcuve et al., 2009), genomic imprinting (Li et al.,

1993), and X-chromosome inactivation (Mohandas et al., 1981). De-regulated DNA methylation leads to human disease, mental retardation, and neurological disorders, such as Rett Syndrome (Warren, 2007; Jin et al., 2008; Delcuve et al., 2009). Epigenetic reprogramming of primordial germ cells is crucial for erasing inherited epigenetic marks; and in the establishment of totipotency (Reik et al., 2001). Shortly after fertilization, the male pro-nucleus within the zygote will go through a process of genome-wide active and passive demethylation events, followed by *de novo* methylation (Santos et al., 2002; Delcuve et al., 2009). How cytosine methylation exerts a gene silencing effect is contributed to a couple of scenarios. Firstly, transcription factors may have difficulties in binding nearby methylated DNA, due to the structural hindrances associated with methylated CpG sites (Watt and Molloy, 1988; Tate and Bird, 1993). Secondly, repressor protein complexes and methyl-CpG-binding proteins (MBD) can directly bind to methylated cytosines, preventing transcription initiation (Hendrich and Bird, 1998; Wade, 2001; Zachariah and Rastegar, 2012). The primary identified member of the MBD proteins is the methyl-CpG-binding protein 2 (MeCP2; Meehan et al., 1992), mutations in which lead to Rett Syndrome (Amir et al., 1999). MeCP2 is an interesting protein, as it can bind to both methylated and non-methylated DNA and act as either a transcriptional repressor or an activator (Chahrour et al., 2008; Delcuve et al., 2009; Zachariah and Rastegar, 2012). In the brain, direct association of MeCP2 with constitutive and facultative heterochromatin marks depends on DNA methylation (Thambirajah et al., 2011), and its genomic localization follows DNA methylation (Skene et al., 2010). While MeCP2 is a widely expressed nuclear protein and can be found in multiple tissues and cell types, it is particularly important in the maturation of the central nervous system (CNS) and plays key roles in neuronal maturation and dendrite formation (Rastegar et al., 2009; Squillaro et al., 2012; Zachariah and Rastegar, 2012). MeCP2 is involved in many cellular pathways, acting both as an activator or a repressor, depending on the specific proteins with which it may interact (Chahrour et al., 2008). Its direct and indirect interacting protein partners include HDAC1, HDAC2, the transcriptional repressors mSin3A, and CoREST (Jones et al., 1998; Nan et al., 1998; Ballas et al., 2005). The enzymes responsible for 5mC are DNA methyltransferases (DNMTs), which consist of DNMT1, acting during replication, and DNMT3a and DNMT3b in *de novo* circumstances (Okano et al., 1999; Bestor, 2000; Delcuve et al., 2009; Zachariah and Rastegar, 2012). There are pivotal crosstalks between 5mC and histone PTM (Fuks, 2005; Cedar and Bergman, 2009), which for example, are evident in heterochromatin with high levels of H3K9me3 equating with 5mC (Stancheva, 2005). Also, the recruitment of HDAC to CpG sites augments the repression of genomic loci (Dobosy and Selker, 2001). These epigenetic conjunctions are apparent throughout the epigenome, and consequently, should be taken into consideration when investigating the epigenetic regulation of any given gene.

First discovered and reported in phage DNA over half a century ago (Wyatt and Cohen, 1953), the 5-hydroxymethylcytosine (5hmC) is recognized as the "sixth base" of the genome. The 5hmC is an epigenetic mark found directly on DNA, with specific tissue (Nestor et al., 2012) and unique distribution pattern during mammalian development (Tahiliani et al., 2009; Ruzov et al., 2011).

Within the paternal pro-nucleus of the post-fertilized zygote, the global loss of 5mC, coincides with genome-wide gain of 5hmC (Ruzov et al., 2011). Through the action of TET (10–11 Translocation) proteins, 5mC is converted into 5hmC (Ito et al., 2010). Although the role of 5hmC is still not fully clear, it is reported to have relatively higher expression in pluripotent stem cells, multipotent stem cells, and within the CNS (Ruzov et al., 2011). In the cerebellum, 5hmC is more enriched in the Purkinje cells compared to the granular cells, being reported to be at about 40% of the 5mC marks highlighting a cell type-specific role for 5hmC (Kriaucionis and Heintz, 2009). Nuclear proteins that bind to 5mC, such as MeCP2 or MBD do not interact with 5hmC (Huang et al., 2010), and the bisulfite sequencing technique that has been used commonly for the determination of DNA CpG methylation does not discern between 5hmC and 5mC (Munzel et al., 2011). As a result, this may reinforce a re-validation of our current knowledge about known methylated sites attributed with specific genomic localities obtained with classical bisulfate sequencing. It is recently shown that the TET enzymes not only convert 5mC into 5hmC, but they are also capable of further oxidization into formylcytosine (5fC) and 5 carboxylcytosine (5caC; Ito et al., 2011). The presence of 5fC and 5caC have been seen in murine ESC (Ito et al., 2011; Pfaffeneder et al., 2011), however, the functional role of these novel cytosine marks have not been fully uncovered yet.

#### MICRO RNA

Apart from the role of chromatin structure, histone PTM and DNA marks, another form of epigenetic regulation involves RNA molecules that are affiliated with epigenetic memory (Morris, 2009). MicroRNAs (miRNAs) are ~22 nucleotide-long Non-coding RNA molecules that can regulate gene expression *via* binding to the messenger RNA with anti-sense sequence homology, leading to transcript degradation or translational inhibition, thereby adversely impacting protein expression (Bartel, 2004). MicroRNAs are critical in the epigenetic regulation and they have been shown to participate in the establishment of *de novo* DNA methylation in murine ESC (Sinkkonen et al., 2008), with capability to modulate chromatin structure and key histone modifiers (Chuang and Jones, 2007). MicroRNAs have been elucidated to play regulatory roles in stem cell proliferation and differentiation (Dubois-Dauphin et al., 1991) and loss of components in the miRNA pathway, such as Dicer, can alter these processes (Bernstein et al., 2003). It is the coordinated pattern of DNA marks and histone modifications, along with miRNA activities, which allows the proper differentiation of fully functional cell types in the CNS, and throughout the body.

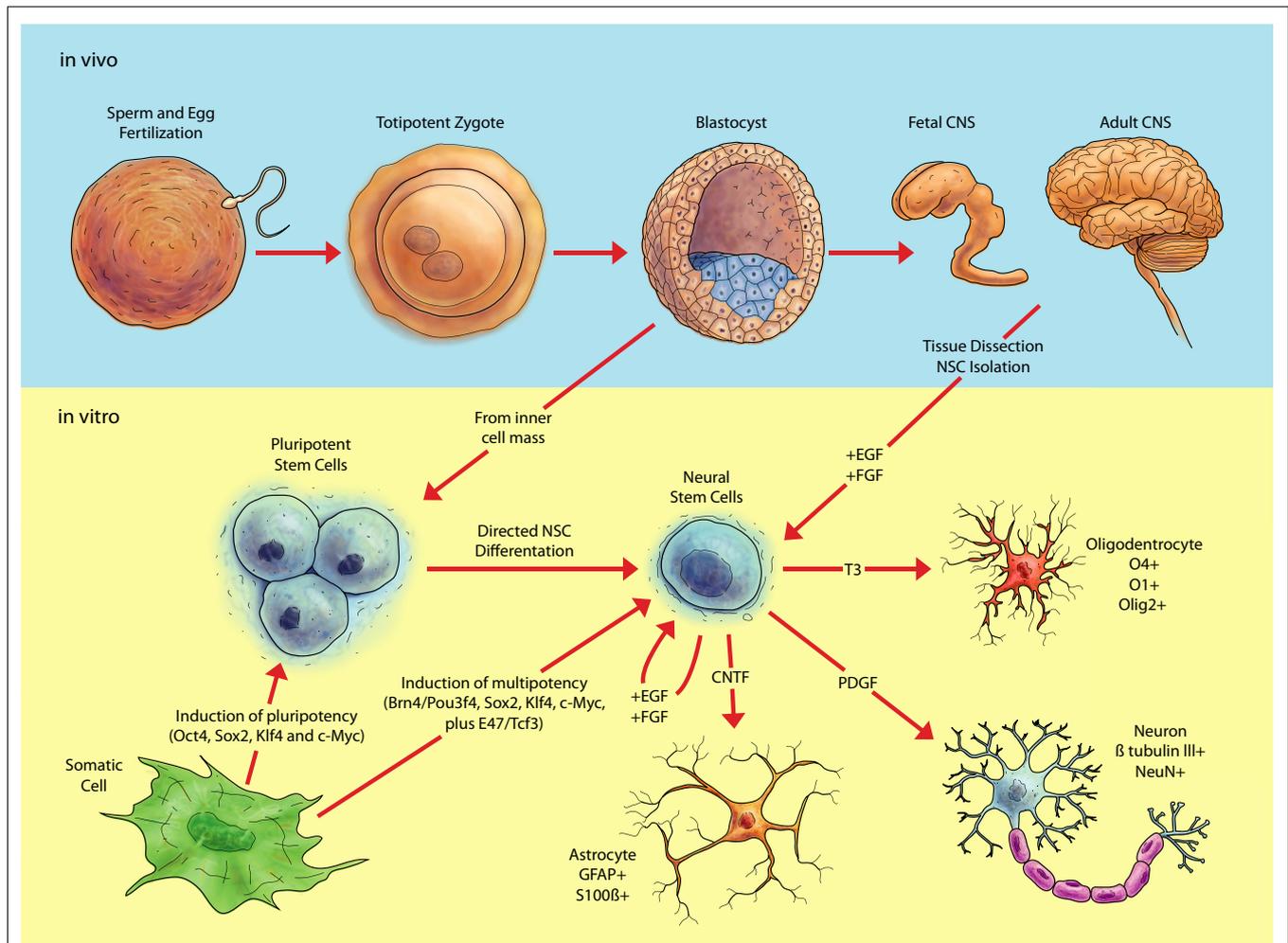
#### CENTRAL NERVOUS SYSTEM DEVELOPMENT; THE ROLE OF HOMEBOX GENES

As embryonic development proceeds, the blastocyst will form three main layers namely: the mesoderm, the ectoderm, and the endoderm. It is from the ectoderm that nervous tissue arises, through a tightly regulated process. The early embryo consists of the neural tube, which will eventually become the brain and the spinal cord with full development of the CNS. In each tissue lineage, multipotent stem cells exist which have a more restricted range of cells to become; in the nervous system, these would be

neural stem cells (NSC; Temple, 1989; Merkle and Alvarez-Buylla, 2006; **Figure 1**). Within the CNS, there is an emerging focus on molecular mechanisms of extracellular signals translating into “epigenetic” modifications. In this regard, the activities of homeobox transcription factors exemplify the intricately coordinated network of developmental control.

In the developing embryo, NSC sense and respond to signals that influence anterior–posterior (AP) and dorso-ventral (DV) axis of the CNS (Rubenstein and Beachy, 1998; Hoch et al., 2009). During development, NSC give rise to a wide range of cell types and neurons within the CNS along the body axis. It is fascinating how NSC can generate particular neurons in specific locations with appropriate extensions projecting toward target tissues and properly reaching other neurons. A major part of these instructions are dictated by the expression of a large family of genes with a common DNA sequence called the “homeobox (HB),” a 180-bp DNA sequence, encoding for a DNA-binding domain called the “homeodomain” (HD). The homeodomain was discovered in the fruit fly “*Drosophila Melanogaster*” about three decades ago as a highly conserved DNA-binding domain found within the developmentally important genes (McGinnis et al., 1984). These genes were referred to as the “*homeotic genes*,” currently known as the “*Hox genes*” (Barber and Rastegar, 2010). Since then, many HD-containing proteins have been characterized in mammals sharing a similar 60 amino acid-long DNA-binding domain. Eight such *Hox* genes exist in fruit fly, and 39 *Hox* genes are identified in mammals. *Hox* genes are key regulators of the hindbrain segmentation into rhombomeres (r) and the “*Hox code*” expression within each rhombomere instructs the cellular composition of each individual hindbrain segment. The hindbrain, that is also named the “*rhombencephalon*,” is the most posterior compartment of the brain in a developing embryo, and anterior to that we find the midbrain followed by the forebrain (Tvrdik and Capecchi, 2006; Narita and Rijli, 2009). *Hox* genes are master regulators of development and cellular differentiation that determine the identity of individual cells within the AP and dorso-ventral axes of the hindbrain including segmental regulation within the CNS. *Hox* genes will further dictate and direct the cellular migration, axonal direction and the process of somatogenesis during embryogenesis and development (Studer et al., 1996; Gavalas et al., 1997; Gaufo et al., 2004; Oury et al., 2006; Oury and Rijli, 2007).

The action of homeobox transcription factors is mediated *via* regulation of their downstream target genes. As a result, HOX proteins will determine the precise time and space of any given cell type to develop and differentiate along the AP axis of a developing embryo during embryogenesis. *Hox* genes will further control the proper function of each developed organ within the body. Such a precise action of *Hox* genes requires their tight control of expression at the exact location during development and in adulthood. The mis-regulation of *Hox* genes leads to human disease and cancer (Barber and Rastegar, 2010). HOX proteins also regulate the differentiation of the AP and transverse planes of the spinal cord through a collinear expression in a temporal and spatial activation order with the genes located 3′ of each cluster expressed earlier and more anteriorly compared to the more 5′ genes within the same cluster. *Hox* gene expression in the hindbrain is controlled by different factors and stimuli including retinoic acid



**FIGURE 1 | Pathway of pluripotent stem cells to neural cell populations.** Fertilization and subsequent cellular divisions create the embryonic blastocyst, where pluripotent ESC are derived from the inner cell mass; ICM). Additionally, pluripotent and multipotent-like cells can be created *via* transduction of various factors into differentiated tissue, such as fibroblasts. *In vitro* analyses of pluripotent and multipotent neural stem cells are integral for understanding aspects of

neural differentiation. The *in vivo* niche of stem cells contains a considerable diversity of biomolecules whose roles still need to be deciphered. Exposure of ESC *in vitro* to various growth factors in serum free media such as fibroblast growth factor 2 (FGF2) and epithelial growth factor (EGF) allows selection of cell lines possessing a neural fate. Neural stem cells can also be acquired from adult tissue and expanded *in vitro*.

(RA), a “vitamin A”-derivative, which functions in the embryonic anterior-posterior patterning. RA treatment has been used to study the transcriptional expression of *Hox* genes *in vitro* (Rastegar et al., 2004; Kobrossy et al., 2006; Nolte et al., 2006). The response to RA is mediated through retinoic acid response elements (RARE), which have been identified and characterized in the regulatory elements of many *Hox* genes including *Hoxa1*, *Hoxb1*, *Hoxa4*, *Hoxb4*, and *Hoxd4* (Langston and Gudas, 1992; Marshall et al., 1994; Studer et al., 1994, 1994; Dupe et al., 1997; Gould et al., 1998; Packer et al., 1998; Zhang et al., 2000; Huang et al., 2002; Rastegar et al., 2004; Nolte et al., 2006). There are auto- and cross-regulatory loops that control the transcriptional expression of *Hox* genes, examples of which are characterized in the expression of the most 3' gene within the B cluster (*Hoxb1*) within the rhombomere 4. *Hoxb1* expression in r4 is controlled by a network of signaling molecules,

involving *Hoxb1*, *Hoxb2*, *Hoxa1*, and *Hoxa2* (Tumpel et al., 2007; Barber and Rastegar, 2010).

HOXB1 regulates the development of the VII facial nerve of the peripheral nervous system, which derives from r4 and has a transient expression prior to the formation of r5, r6, and r7 (Trainor and Krumlauf, 2001; Tumpel et al., 2009). *Hoxb1* loss-of-function in mice indicates a non-autonomous role for *Hoxb1* in facial nerve development, delineating an essential function in controlling facial branchiomotor neuron development at the level of r4 in the hind-brain (Arenkiel et al., 2003). The cranial neural crest cells are highly dynamic and generate complex derivatives such as cartilage, bone, muscle, glands, neurons, and glia and represent a population of stem cells with restricted cellular commitments (Cornell and Eisen, 2005; Trainor, 2005; Schlosser, 2008). *Hoxb1* expressing neural crest cells preferentially generate glial progenitors associated with

the development of motor component of VII facial nerve complex and are destined to become peripheral glial cells (Arenkiel et al., 2003). In addition to their role in AP embryonic patterning and neurogenesis, *Hox* genes are also involved in different types of human cancer such as leukemia (Slany, 2009), breast cancer (Duriseti et al., 2006), and brain tumors (Abdel-Fattah et al., 2006). HOX proteins regulate the expression of their target genes through DNA-binding with different partners, influencing their binding activity and specificity (Huang et al., 2005; Barber and Rastegar, 2010). The best-characterized HOX cofactors are the proto-oncogene Pre-B cell leukemia transcription factor 1 (PBX1) and murine ectopic integration site (MEIS); both affecting HOX DNA-binding activity/specificity (Huang et al., 2005; Barber and Rastegar, 2010). HOX proteins have been the subject of intensive research studies during embryonic development (Rastegar et al., 2004; Stern et al., 2006), ESC neurogenesis (Morey et al., 2007), and embryonic carcinoma cell (ECC) differentiation (Rastegar et al., 2004; Huang et al., 2005; Kobrossy et al., 2006; Nolte et al., 2006). However, due to the redundancies, which exist between the members of each paralog groups, our knowledge about their downstream target genes, besides *Hox* genes themselves, is currently very limited.

### PLURIPOTENT EMBRYONIC STEM CELLS

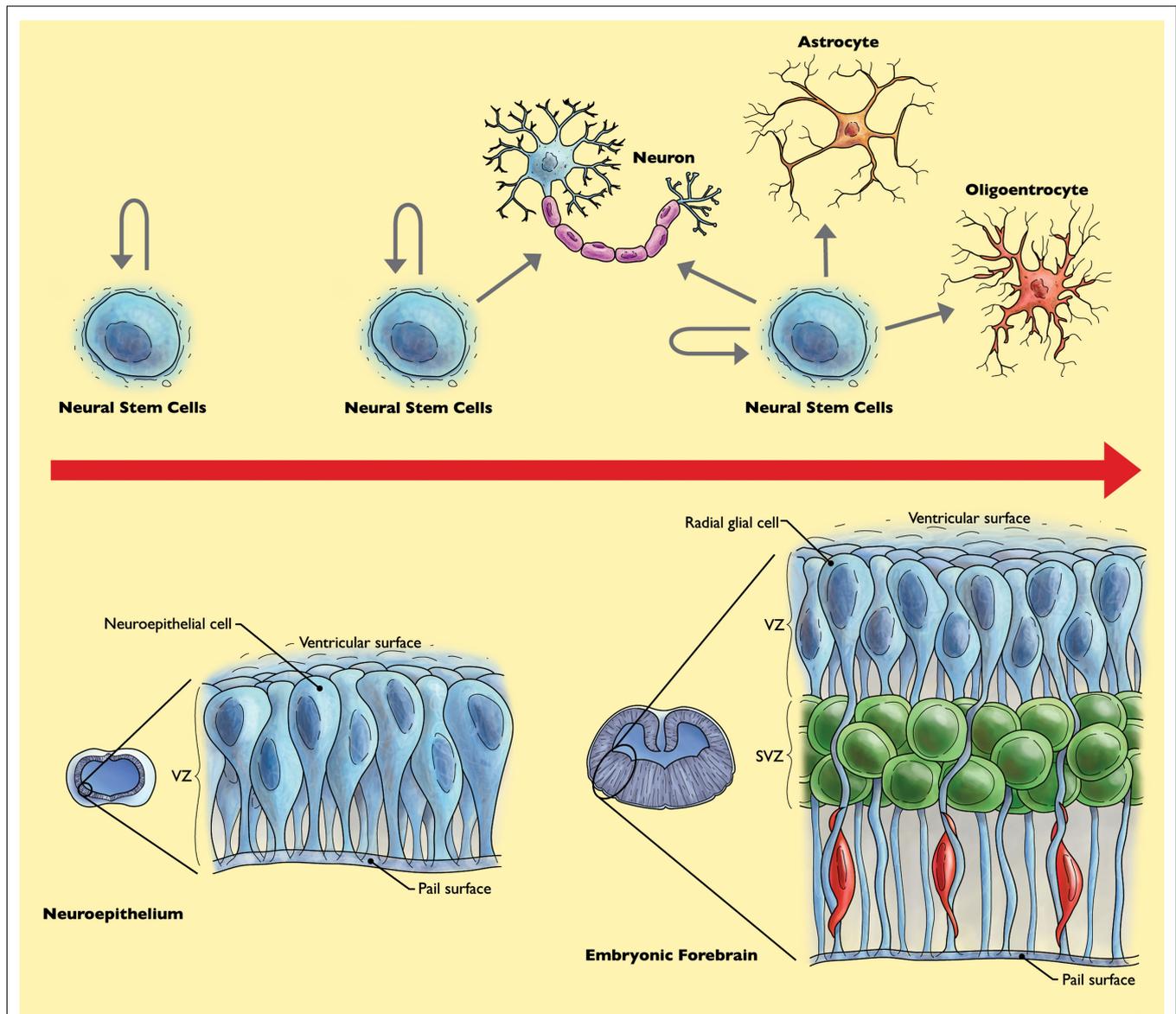
Following the unionization of an egg and a sperm, a single fertilized totipotent zygote will be formed that is capable of developing into all embryonic and extra-embryonic tissues. The resulted zygote will subsequently divide many times toward the generation of a sphere-shaped conglomerate, known as the “blastocyst” (Rossant and Tam, 2009). This early embryonic structure has an outer layer called the trophoblast, which will form the placenta, and inside resides the ICM (Rossant et al., 2003), where ESCs can be isolated (Figure 1). ICM-derived ESCs are pluripotent with the potential of indefinite self-renewal and ability to differentiate into any cell type and tissue within the body (Reubinoff et al., 2000; Odorico et al., 2001; Rastegar et al., 2011). Herein lays ethical controversy in stem cell research, as harvesting cells of the ICM will resultantly destroy the embryo; one of the reasons why alternative methods for acquiring pluripotent cells are eagerly being sought recently (Takahashi and Yamanaka, 2006; Robinton and Daley, 2012). Perhaps one of the most achievements in the field, is the *in vitro* generation of pluripotent stem cells termed “induced pluripotent stem (iPS) cells” from fibroblast cells in both mice and humans. Shinya Yamanaka was the first to demonstrate that retroviral transduction of cDNA comprising the pluripotent factors, *Oct4*, *Sox2*, *Klf4*, and *c-Myc*, can transform mature, lineage restricted somatic cells into de-differentiated cells with ESC-like properties (Takahashi and Yamanaka, 2006; Takahashi et al., 2007). Since then, many labs have successfully generated iPS cells from different cell types and with different inducing techniques (Wernig et al., 2007; Aoi et al., 2008; Okita et al., 2008; Park et al., 2008; Hotta et al., 2009), such as the use of piggy back vectors (Woltjen et al., 2009), recombinant proteins (Zhou et al., 2009), nanotechnology (Ruan et al., 2011), and small chemical compounds (Huangfu et al., 2008; Shi et al., 2008). Recently, the transcription factors, *Brn4/Pou3f4*, *Sox2*, *Klf4*, *c-Myc*, plus *E47/Tcf3*, have been used to directly induce neural stem-like multipotent stem cells from

murine fibroblasts (Han et al., 2012). In general, the processes of re-programming the nuclear transcriptome would synchronize with transformation of the epigenome (Sasaki and Matsui, 2008; Fussner et al., 2011b; Mattout et al., 2011). Overall, the generation of iPS cells has brought great promise and excitement into the translational research, regenerative medicine and modern innovative therapeutic approaches with regards to addressing the current shortage of organs and tissues for transplantation applications. Currently, there is an intensive effort in our scientific community in search of perfecting the iPS cell generation and to better develop technologies for *in vivo* application of iPS cells. Regardless, this technology has already given us an opportunity to model human disease such as neurological disorders, with no accessible neurons to study the mechanism of disease prior to the development of the iPS technology. Although iPS cell research is still at its infancy period, it has already given us hope by the success of curing limited diseases in mouse models, such as sickle cell anemia in mice (Hanna et al., 2007).

To maintain pluripotency, ESCs continuously express key transcription factors such as OCT4 and NANOG along with the SOX2 protein during their self-renewal. These proteins contribute and share at least in part in the regulation of downstream developmentally important genes. Additionally, OCT4 controls the expression H3K9me2/3 demethylases and as a consequence contributes in maintaining the epigenetic landscape during ESC self-renewal (Loh et al., 2007; Calvanese and Fraga, 2012). Moreover, through the simultaneous existence of both active and inactive histone marks (referred to as “bivalent marks,” which will be discussed later) on the specific developmentally regulated genes, they would remain at the poised state of transcription during ESC self-renewal. Subsequently, during differentiation, these genes may lose one type of marks and become active or silenced depending on the direction of differentiation. In general, chromatin structure in ESCs is highly dynamic (Meshorer et al., 2006) and during differentiation, histone modifications of open chromatin such as histone H3 and H4 acetylation are reduced globally, with a general increase in the repressive marks such as H3K9me3. This leads to more condensed chromatin at specific genomic loci (and corresponding genes) for proper differentiation (Lee et al., 2004; Meshorer et al., 2006). Chromatin condensation, in part, is controlled by linker histone H1 with a high ratio close to 1/nucleosome in differentiated cells and 0.5 in ESCs. Depletion of three histone H1 isoforms is lethal in mice, but such ESCs (Triple histone H1 Knock Out) with 0.25 histone H1/nucleosome, have generated perfect tools and opportunity to study the role of chromatin compaction in controlling specific genomic loci (Fan et al., 2005).

### MULTIPOTENT NEURAL STEM CELLS

The human brain is a central part of our nervous system, with a constitution of over one-hundred billion neurons, with an average of 7000 connections for each neuron (Drachman, 2005). This large number of neurons seems to be able to compensate for the loss of activity in part of the cells in the brain. However, depending on location, neuronal activity loss may have severe consequences leading to neurological disorders. In this respect, NSC have attracted much attention because of their potential to replace neurons and glia which are lost or damaged in trauma, stroke, spinal cord injury,



**FIGURE 2 | Behavior of neural stem cells (NSC) throughout forebrain development.** Early during development, NSC increase their population of stem cells through symmetrical cell divisions. At mid-gestation, at the “neurogenic phase,” NSC divide asymmetrically, yielding a NSC and a neuron

or neural progenitor cell and neuron. Neural progenitor cells can further divide symmetrically to produce two neurons. During the “gliogenic phase” in late gestation, populations of not only neurons, but astrocytes and oligodendrocytes are created (Merkle and Alvarez-Buylla, 2006).

amyotrophic lateral sclerosis (ALS), Parkinson’s, or Alzheimer’s disease. Altman (1962) observed and reported the incorporation of tritiated thymidine in neural cell types within traumatized regions of rat brains. However, it took many years, with key experimental evidence to prove to the scientific community that the adult nervous system can produce new neurons. There is now a wider acknowledgment that neurons are being produced from NSC not only during embryonic development, but also in the postnatal brain. In the developing embryo, NSC sense, and respond to signals that influence the AP and dorso-ventral axes of the CNS (Rubenstein and Beachy, 1998; Hoch et al., 2009). NSC are primary progenitors capable of self-renewal and differentiation into the

three main cell types of the mammalian nervous system; neurons, astrocytes, and oligodendrocytes (Namiyama et al., 2008). During the progression of nervous system development, neural stem cell will behave differently while dividing, with respect to the particular fate commitment of their daughter cells (Hirabayashi and Gotoh, 2005). During early gestation, NSC exist as bipolar neuroepithelial cells across the neural tube (Figure 2). At this stage NSC self-renew and symmetrically divide into two identical daughter cells, similar to the parental cell to increase the number of NSC. Later in development and during mid-gestation, NSC will divide asymmetrically, producing a new NSC and a neuron, or a neural stem cell and a neural progenitor cell. Neural progenitor (or precursor)

cells are intermediary between the stem cell and more differentiated cells and have more limited self-renewal capability. However, the distinction between neural precursor cells and NSC are not always clearly defined in experimental analyses, and the terms are often used interchangeably. In later stages of embryonic development, NSC are capable of both self-renewal and differentiation and may give rise to neurons, astrocytes, and oligodendrocytes (**Figure 2**; Qian et al., 2000).

As mentioned earlier, functional neurogenesis is not limited to embryonic development and will continue throughout life (Ma et al., 2010) even in the adulthood (Altman, 1962; Goldman and Nottebohm, 1983; Eriksson et al., 1998). In the adult mammalian brain, NSC reside in the sub granular zone of the dentate gyrus in the hippocampus, and in the sub ventricular zone of the lateral ventricles (Aimone et al., 2010; Gage, 2010; Bonfanti and Peretto, 2011; Lee et al., 2012). Adult neurogenesis has been shown to be modulated by a wide range of influences from exercise (van Praag et al., 2005; Brandt et al., 2010) and learning (Sultan et al., 2010; Anderson et al., 2011), to pharmacological treatment with anti-depressants (Duman et al., 2001; Anacker et al., 2011). As a result of these observations, there is promise for discovering new therapeutic modes of neural induction, for those who have lost or damaged neural tissue. Since epigenetic activities are critical for cellular differentiation (Schofer and Weipoltshammer, 2008; Delcuve et al., 2009; Meissner, 2010), we will briefly discuss the involved molecular mechanisms with development, concerning the transition of embryonic to NSC and mature neural cells, including the recognition of key epigenetic activities.

## EMBRYONIC STEM CELLS EMBARK ON THE NEURAL ROAD

We have discussed about the major epigenetic mechanisms, neural development, ESCs, and the role of NSC in development. We will now focus on the metamorphoses of the epigenome that occurs during neural commitment of ESCs; and briefly address the corresponding extrinsic factors, genetic transcriptional networks, and epigenetic events (Darr and Benvenisty, 2006; Ng and Surani, 2011; Xue et al., 2011).

### EXTRINSIC FACTORS ELICIT THE ACTIONS OF TRANSCRIPTIONAL EQUIPMENT

Embryonic stem cells can be directly differentiated into specially differentiated cell types, yet their default differentiation pathway is into NSC (Smukler et al., 2006). *In vivo*, NSC are isolated from different parts of the CNS both during embryonic development and from the postnatal brain. Extrinsic factors that affect stem cell differentiation include a variety of signaling molecules and growth factors that are critical for maintenance of ESC pluripotency and self-renewal, or triggering differentiation (Pera and Tam, 2010). In case of NSC, the extrinsic factors mainly originate from the cellular niche, referring to the microenvironment where these cells reside within the CNS. In NSC, the extrinsic factors tightly control the maintenance of multipotency of these cells. Important extrinsic factors include the NOTCH Signaling family, Transforming Growth Factor- $\beta$  (TGF- $\beta$ ) superfamily members, Fibroblast Growth Factors (FGF), Neuregulins (NRG), and Platelet Derived Growth Factors (PDGF), to name a few (Mizutani et al., 2007; Pera

and Tam, 2010). EGF and FGF2 are known to regulate neural programming (Reynolds and Weiss, 1996; Kuhn et al., 1997; Palmer et al., 1999), and are used for *in vitro* isolation and expansion of NSC (Gage, 2000; Reubinoff et al., 2001; Abranches et al., 2009; **Figure 1**). One particular member of the EGF family, Beta-cellulin, has been recently shown to promote cell proliferation and drive NSC neurogenesis (Gomez-Gaviro et al., 2012). Interestingly, insulin like growth factors (IGF1 and IGF2) predispose ESCs toward a mesodermal fate (Pieciewicz et al., 2012), yet IGF2 regulates adult hippocampal neurogenesis (Bracko et al., 2012).

### TRANSCRIPTION FACTORS BRIDGE THE STATE OF STEM CELLS

During ESC self-renewal, key transcription factors such as OCT4, NANOG, as well as SOX2 control ESC pluripotency *via* regulating the expression of specific downstream target genes (Heng and Ng, 2010). Recently, other proteins are also shown to have similar roles including TAF3, E-cadherin, KLF4, and PBX1 (Chan et al., 2009; Liu et al., 2011a; Redmer et al., 2011). To point out another level of control to these factors, the microRNA, mir-145, can target the 3' untranslated region of *Oct4*, *Sox2* (and *Klf4*) transcripts; and up-regulation of mir-145 is evident during ESC differentiation (Xu et al., 2009). The zinc finger nuclear protein, ZFP521, has been recently shown to be a key regulator for driving neural differentiation of ESCs; activating early neural genes in association with the co-activator p300 (Kamiya et al., 2011). In NSC, the SOXB1 and GLI families, CBF-1, HMGA2, HES1/5, BMI-1, HESR1/2, TLX, MASH1, PAX6, and REST have been associated with self-renewal, growth, and differentiation (Ahmed et al., 2009). There is ultimately a cascade of epigenomic re-organization from the extrinsic cues to the genomic machinery for modulating the stem cell state.

### GENERAL CHANGES IN GENOMIC ACCESSIBILITY DURING LINEAGE COMMITMENT

The chromatin structure in ESCs is relatively flexible and dynamically open with particular epigenetic signature, having a "closure" in many regions as ESCs commit to a particular lineage (Delcuve et al., 2009; Rastegar et al., 2011). This is valid for genes associated with differentiation, being "silenced" by DNA methylation and inactive histone marks in pluripotent cells compared to multipotent stem cells (Aranda et al., 2009; Barber and Rastegar, 2010; Rastegar et al., 2011). For example, during the transition of ESCs into the three germ layers, there is an increase in number of common DNA promoter methylation regions (CpG sites; Delcuve et al., 2009; Isagawa et al., 2011; Rastegar et al., 2011). Both histones (H1, H2B, H3) and non-histone chromatin proteins, such as the heterochromatin protein 1 (HP1) are shown to be loosely affiliated with chromatin structure in ESC, leading to a plastic nature of pluripotent stem cells compared to more lineage committed cells (Meshorer et al., 2006). Further, higher global levels of histone acetylation are detected in pluripotent stem cells in contrast to multipotent, and tissue specific stem cells as well as somatic cell types (Efroni et al., 2008). These findings complement the observation that histone de-acetylase activity is required for ESC differentiation (Lee et al., 2004). Pluripotent ESC also possesses high levels of transcriptional activity, yet a large proportion of gene transcripts are only expressed at low levels. Based on a variety of global transcript analyses, it is hypothesized that as

ESCs commit toward a specific lineage, downregulation of many different gene transcripts increases the effectiveness of other genes whose transcripts are not downregulated (Bibikova et al., 2008). As a unique epigenetic characteristic of ESCs; possessing a “poised” transcriptional state or bivalent chromatin marks are detected at many developmentally important genomic loci such as the *Hox* clusters (Bernstein et al., 2006; Delcuve et al., 2009; Barber and Rastegar, 2010).

#### THE ROLE OF BIVALENT HISTONE MARKS DURING PLURIPOTENCY AND DIFFERENTIATION

Transcriptionally active genes in ESCs, such as *Oct4* or *Nanog* are characterized by histone acetylation and histone H3-Lysine 4 tri-methylation. In the other hand, most key developmental genes remain inactive during ESC self-renewal carrying both “active” and “inactive” histone marks. This has led to the simultaneous detection of histone H3-lysine 27 tri-methylation and lysine 4 methylation, referring to as the category of “bivalent chromatin” marks. Among genes with bivalent chromatin marks are the master regulators of embryonic development, namely the members of all four *Hox* clusters (Barber and Rastegar, 2010). As described previously, HOX homeoproteins are highly conserved transcription factors that instruct the AP axes of the developing embryo (Rastegar et al., 2004; Huang et al., 2005; Kobrossy et al., 2006; Nolte et al., 2006; Barber and Rastegar, 2010). The 39 mammalian *Hox* genes are organized in four clusters A–D, in four different chromosomes with unique collinear gene activation across each chromosome (Rastegar et al., 2004; Barber and Rastegar, 2010) along the AP axis. It has been suggested that the underlying mechanism is a unidirectional chromatin opening and de-condensation along each chromosome, resulting in precise expression pattern that is corresponding to *Hox* physical position within each cluster, the phenomenon which is called “colinearity” (Rastegar et al., 2004; Barber and Rastegar, 2010). We have shown such unidirectional chromatin opening at the *Hoxd4* locus during embryonic development and in differentiated P19 ECCs (Rastegar et al., 2004; Barber and Rastegar, 2010). HOX proteins play key roles in ESCs differentiation and their transcriptional expression is tightly regulated by histone marks, chromatin condensation, other transcription factors (Chambeyron and Bickmore, 2004; Chambeyron et al., 2005; Huang et al., 2005; Kobrossy et al., 2006; Nolte et al., 2006), and non-coding microRNAs (Barber and Rastegar, 2010).

During differentiation of pluripotent ESCs, the “poised” state essentially readies the stem cells for multiple descendancies; as losing one of the bivalent marks can lock a gene at a “off” or “on” state of transcription. Perhaps the most well characterized examples of developmentally important genes with these bivalent chromatin marks are the *Hox* genes. The Polycomb Repressive Complexes (PRC) play a role in regulating the state of ESCs (Boyer et al., 2006) as PRC1 can compact chromatin and catalyze ubiquitination of histone H2A, whereas PRC2 can aid in compaction as well as tri-methylation of H3K27 (Delcuve et al., 2009; Margueron and Reinberg, 2011; Rastegar et al., 2011). PRC2 is associated at genes necessary to preserve a pluripotent state (Lee et al., 2006), but it has been now challenged whether the member PRC2 is actually necessary to maintain ESC pluripotency (Chamberlain et al., 2008). More recently, a component of PRC2, Polycomb-like

3 (Pcl3), has been shown to be instrumental for ESC self-renewal by mediating PRC2 binding, promoting H3K27me3 formation (Hunkapiller et al., 2012). Also, the Polycomb family repressor, Bmi-1, is important for neural stem cell self-renewal (Molofsky et al., 2003). Overexpression of Bmi-1 in *in vitro* cultures of NSC correlates with the increased proliferation and neurogenesis; however, this was not reflected with the *in vivo* overexpression in a transgenic murine model (He et al., 2009). The Trithorax Group (TrxG) counteracts the Polycomb Group’s repressive effect on histone bivalency (Schuettengruber et al., 2011) with tri-methylation of H3K4 accomplished by mixed lineage leukemia (MLL) of the Trx group (Wang et al., 2009).

Bivalent marks may become univalent during neural differentiation; securing the epigenetic identity of a particular genomic region (Delcuve et al., 2009). The MLL member, MLL1, has been shown to be integral for resolving bivalent loci in NSC and during neurogenesis (Lim et al., 2009). A specific demethylase of H3K27me3, JMJD3, acts upon the promoter for *Nestin*, a neurofilament gene important in the process of neural differentiation. This results in the activation of the *Nestin* gene, and a step toward the differentiation of ESCs into NSC (Burgold et al., 2008). Furthermore, the H3K4me2/3 demethylase JARID1B, is essential for ESC commitment toward a neural lineage (Schmitz et al., 2011). Examples of other neural genes that resolve the repressive mark of bivalency as cells commit toward a neural lineage fate commitment include: neurogenins (Ngns), paired box gene 6 (*Pax6*), SRY-Box 1 (*Sox1*), *Sox3*, *Mash1* (achaete–scute complex homolog 1, or *Ascl1*), and NK2 transcription factor related locus 2 (*Nkx2.2*; Mikkelsen et al., 2007; Hirabayashi and Gotoh, 2010). Interestingly, the DNA mark of 5hmC, is prevalent at the promoters of “poised” regions in self-renewing ESCs, and at the genes that are upregulated during differentiation. Therefore, it seems that 5hmC may function by priming regions for transcription as ESCs differentiate (Pastor et al., 2011).

Biomolecules that can be found in niche microenvironments of the developing embryo, or neurogenic regions of the adult CNS, trigger signal transduction pathways leading to intrinsic responses necessary for neural differentiation. Overall, as ESCs ascribe to the neural lineage, non-neural and pluripotency-related genes become silenced and inaccessible for transcription, whereas the promoters and enhancers of neural related genes will become accessible (Hirabayashi and Gotoh, 2010). The NSC, descendants of ESCs, do not possess the plasticity of their parents. In the other words they have a more constricted directional potential, and the cellular identity of their generated daughter cells is in part impacted by the context of the environment in which they reside. Many cells in their niche such as astrocytes, neurons, endothelial cells, ependymal cells, and immature NSC are thought to be crucial components for NSC maintenance and development (Duan et al., 2008). Finally, at the last stages of the developmental journey, NSC will potentially become specialized nerve cells; as neurons are produced during neurogenesis and glia by the process of gliogenesis.

#### NEARING THE END OF THE ROAD: NEURAL DIFFERENTIATION

We have discussed a collection of factors that impact ESC and neural stem cell fate decisions during self-perpetuation and toward lineage differentiation. We will now briefly touch-base on a

selection of genomic machinery and miRNA that are implicated in the processes of gliogenesis and neurogenesis; weighted more toward players in the formation of neurons.

### REST, A FACTOR FOR PREVENTING NEUROGENESIS

First discovered in 2005 (Chong et al., 1995; Schoenherr and Anderson, 1995), the repressor element 1 (RE1) silencing transcription factor (REST), also known as neuron restrictive silencing factor (NRSF), is responsible for silencing neuronal genes in non-neuronal tissues, neural precursor cells, ESCs, and NSC (Chen et al., 1998; Ballas and Mandel, 2005). REST contributes to the regulation of ESC pluripotency, and it is also involved in neural differentiation (Soldati et al., 2012), and controlling ncRNA (Qureshi and Mehler, 2009). The RE1 is another designation for the neuron restrictive silencer element (NRSE), which is found within many neuronal genes (Ooi and Wood, 2007). When cells commit to a neuronal lineage, the REST complex may degrade; resulting in de-repression of its target genes. REST binds to the 21-bp, RE1, and recruits HDACs as well as other chromatin modifiers to silence genes. Furthermore, REST can recruit co-repressor protein complexes such as HDAC1/2, CoREST, and mSin3 (Dewald et al., 2011). Additionally, REST has been observed to mediate the recruitment of PRC1 and PRC2 in certain circumstances (Dietrich et al., 2012).

### MICRO RNAs TARGET NEURAL REGULATORY TRANSCRIPTS

After the discovery of their modulatory role in neural regulation within the last several years (Stark et al., 2005; Krichevsky et al., 2006), miRNAs have been under extensive investigation. One miRNA that is prevalent within the nervous system, and particularly in neuronal cells, is mir-124a (Lagos-Quintana et al., 2002). It has shown to be critical in the process of neural differentiation (Makeyev et al., 2007) specifically in neurogenesis, by targeting *Sox9*, an important player in neural stem cell self-renewal maintenance (Cheng et al., 2009). Recently, in post-ischemic injured rats, mir-124a has been shown to affect *Jag1* levels, as part of the NOTCH signaling pathway. Subsequently, the downregulation of mir-124a induces *Jag1* and proliferation of neural precursor cells, that has been hypothesized to result from the increase of the homeoprotein DLX2, whose corresponding transcripts are targeted by mir-124a (Cheng et al., 2009; Liu et al., 2011b). The nuclear receptor TLX, also plays key roles in neural stem cell self-renewal, and is targeted by mir-9, resulting in increased neuronal production through a negative regulatory feedback loop (Zhao et al., 2009). Expression of mir-125b was similar to mir-124a in that they both increase percentage of differentiated neuroblastoma cells (SH-SY5Y) with neurite outgrowth. Also and some gene targets of the mir-125b have been connected with the negative control of neuronal related genes (Le et al., 2009).

### THE LINK BETWEEN miRNA AND REST

Multiple microRNAs including, mir-9, mir-124a, and mir-132 appear to be controlled by REST (Conaco et al., 2006). For example, mir-9 targets the REST sequence forming a negative feedback loop (Packer et al., 2008). It has been demonstrated that mir-124a directly targets the *SCP1*-3' untranslated region of small C-terminal domain phosphatase 1 (SCP1; Visvanathan et al., 2007).

The phosphatase, is found in non-neuronal tissues and recruited to RE1 by REST, and has anti-neuronal function (Yeo et al., 2005). More recently, mir-26b has been shown to represses the REST cofactor, CTDSP2 to regulate neurogenesis (Dill et al., 2012). Additionally, MeCP2 may interact with REST to suppress particular sites, and MeCP2 itself, is targeted by mir-132 (Klein et al., 2007).

### OTHER NUCLEAR FACTORS IMPLICATED IN NEURONAL REGULATION

In the adult murine hippocampus, GADD45B, is responsible for demethylating the promoter region of genes which are critical for neurogenesis, such as BDNF and FGF (Ma et al., 2009). Nuclear factor kappa B (NFκB) signaling has been observed to have novel functionality by initiating early neurogenesis in NSC (Zhang et al., 2012). Recently, the Querkopf (QKF), a histone acetyltransferase, has been shown to be a useful marker of self-renewal and multipotency in NSC (Sheikh et al., 2012), and it is also essential for normal adult neurogenesis (Merson et al., 2006).

### THE FUNCTION OF STAT-p300/CBP-SMAD IN ASTROCYTE FORMATION

Astrocytic differentiation of NSC can be induced through the activation of the Janus kinase (JAK)-signal transducer and activator of the transcription (STAT) pathway. Cytokines of the interleukin-6 (IL-6) family trigger JAK-STAT, and include the following members; leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), and ciliary neurotrophic factor (CNTF; Bonni et al., 1997). The bone morphogenic protein (BMP) family cytokine members of the TGF-β superfamily activate SMAD transcription factors. Afterward, a complex can be formed between STAT and SMAD proteins by the transcription co-activators, p300/CBP, which possesses histone acetylating functionality (Nakashima et al., 1999). The STAT-p300/CBP-SMAD complex can promote transcriptional activation of associated genomic regions.

Moreover, the Glial fibrillary acidic protein (GFAP) is an astrocytic marker whose promoter region is methylated during early gestation, while it will later become demethylated. Within the promoter of *Gfap*, there is a highly methylated CpG dinucleotide at a STAT binding element when cells are in a neuronal phase. This methylated CpG prevents the STAT-p300/CBP-SMAD complex from facilitating transcriptional activation at the site (Takizawa et al., 2001). In another astrocytic marker, *S100β* it is shown that it has a methylated cytosine in its promoter region, and similarly becomes demethylated later in gestation (Namihira et al., 2004) much like the *Gfap* promoter, allowing the STAT-P300/CBP-SMAD complex to bind and transcription to commence. MeCP2 can also bind 5mC in the exon 1 of *Gfap* and the CpG at the transcriptional start sites of *S100β*, suppressing these astrocytic markers and subsequent differentiation (Setoguchi et al., 2006).

### APPLYING KNOWLEDGE OF THE NEURAL EPIGENOME IN REGENERATIVE MEDICINE

There is much excitement surrounding the application of pluripotent and multipotent stem cells in the hope of replenishing damaged cells and tissues in various medical circumstances, such as in neurodegenerative disease. Clinical trials are currently underway that involve neural stem cell transplantation in patients with ischemic injury and ALS, with preclinical studies being implemented for future cell-based treatments of brain cancer, ischemic

spastic paraplegia, chronic spinal cord injury, and chronic stroke. In order to attain pure populations of differentiated cell types *in vitro* that can be designated for safe therapeutic use, such as differentiated neurons or glia, a thorough comprehension of the epigenetic mechanisms is needed. This is imperative with induced cell types (i.e., iPSCs or induced NSC) where the understanding of re-programmed cells is a bit further than its infancy, as well as other *in vitro* cultured cell types. Nonetheless, patient-specific stem cells hold great promise in regenerative medicine by supplying patients with genetically identical matches for treatment, although heed must be taken to ensure that any molecular variances (with epigenome, proteome, etc.), if any, do not perturb their potential benefit. Therefore, we believe that a comprehensive mapping of the epigenome would be invaluable for this cause; with further investigation of novel marks such as 5hmC, histone variants, and factors contributing to their presence.

## CONCLUSION

A selection of key epigenetic events studied in neural stem cell differentiation have been discussed, including novel findings to date as well as more established results in the field. Overall, ESCs can be described as having a dynamic increase of “closed” chromatin marks, and decrease of “open” chromatin marks during lineage commitment, with unique modification patterns attributed to

lineage orientated genes. To travel further down the neural path, a resolution of bivalent marks is required for stem cells. The differentiation of NSC into neurons or glial cells requires regulation of multiple miRNAs including mir-124a, mir-125b, mir-26b, and epigenetic modifying transcriptional machinery, such as Gadd45b, QKF, REST, or STAT-p300/CBP-SMAD complexes. These examples are only a few of many that demonstrate the elaborate organization of the epigenome in ESCs as they take the journey to become neural cell types.

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