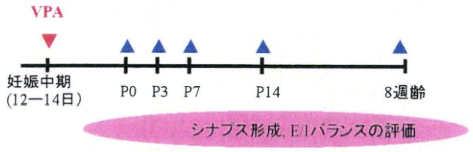
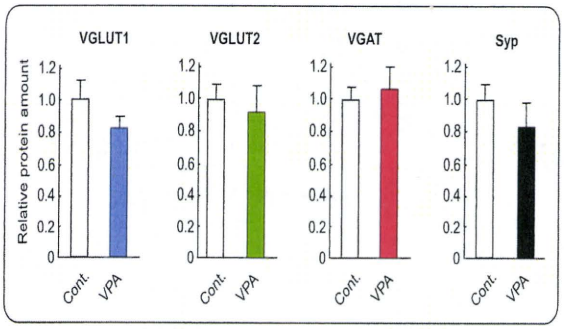
*in vivo*暴露

対照群

VPA投与群 (300mg/kg)

妊娠マウス♀への経口投与





妊娠中期にバルプロ酸を経口投与し、産仔海馬におけるシナプスマーカー及びE/Iバランスマーカーの発現量を解析した。

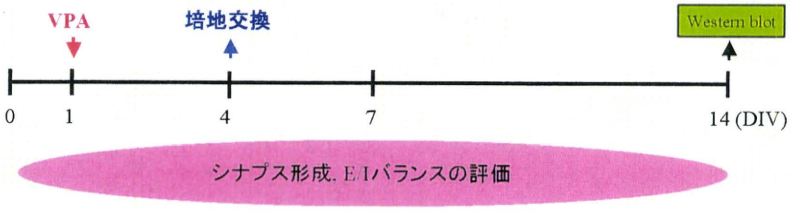
産仔の成熟期にVGLUT1とSynaptophysinの軽微な減少傾向が見られたものの、E/Iバランスの顕著な破綻は見られなかった。

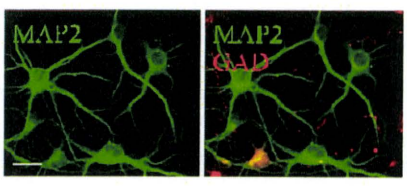
実験方法

P1由来大脳皮質神経初代培養

対照群 (0 mM VPA)

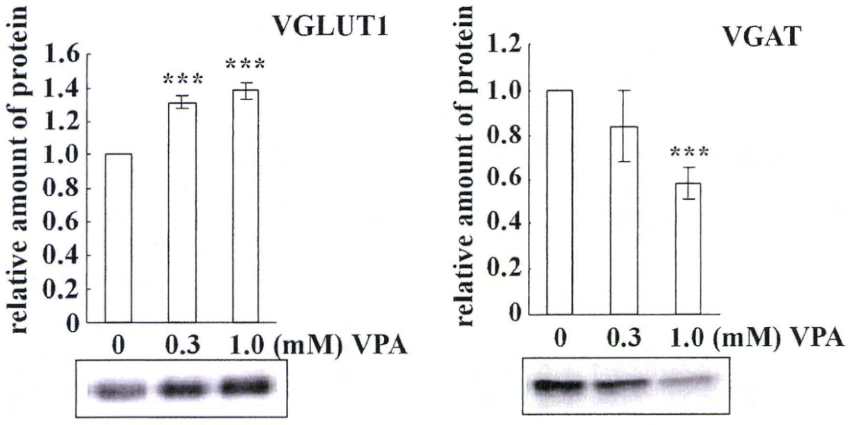
VPA投与群 (0.3 mM, 1.0 mM)



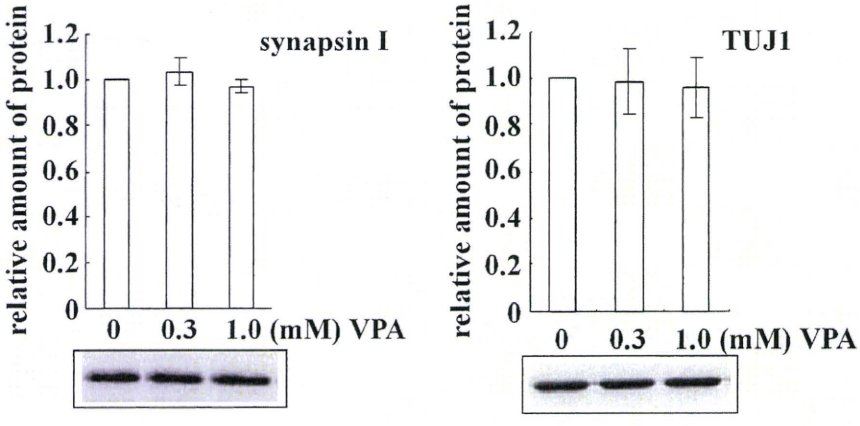


DIV4の海馬神経初代培養

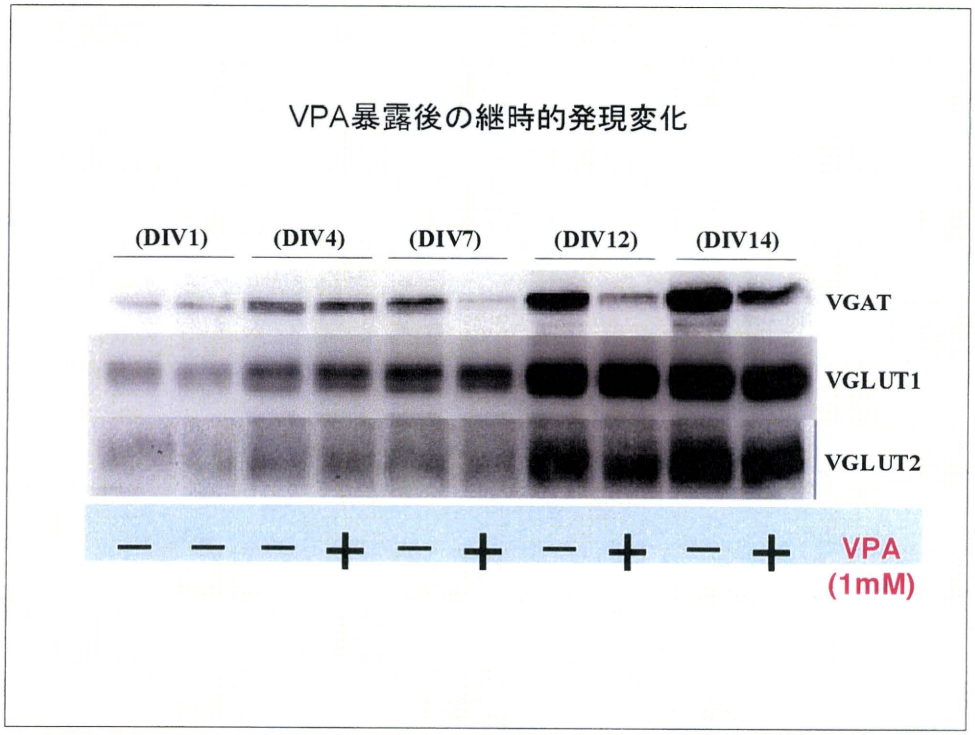
VPA暴露によりE/Iバランスが E に傾く

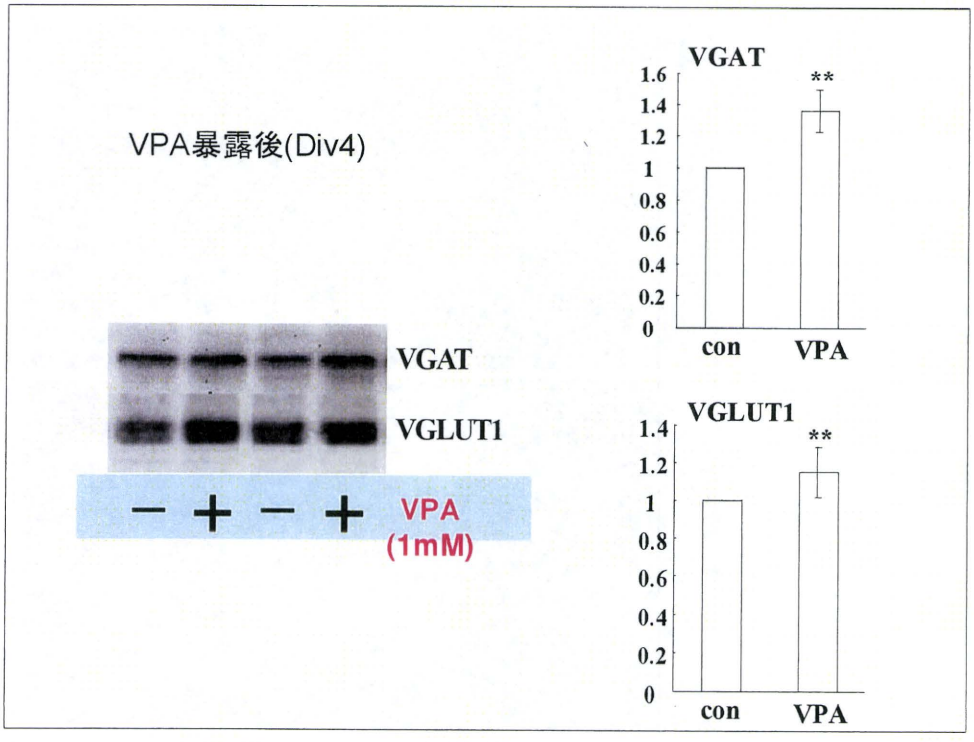


VPA暴露により神経細胞数や シナプス数に変化はない



プレシナプスマーカー　　幼若神経細胞マーカー


VPA暴露後の継時的発現変化
(DIV1)
(DIV4)
(DIV7)
(DIV12)
(DIV14)
VGAT
VGLUT1
VGLUT2
− − − + − + − + − +
VPA
(1mM)

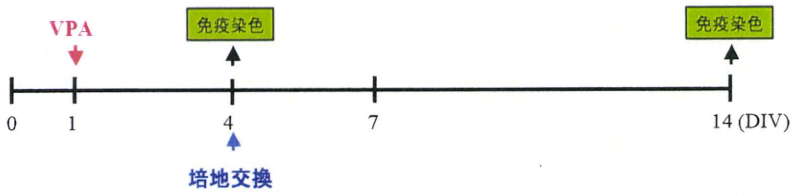

VPA暴露後(Div4)
VGAT
VGLUT1
− + − +
VPA
(1mM)
VGAT
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
con
VPA
VGLUT1
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
con
VPA

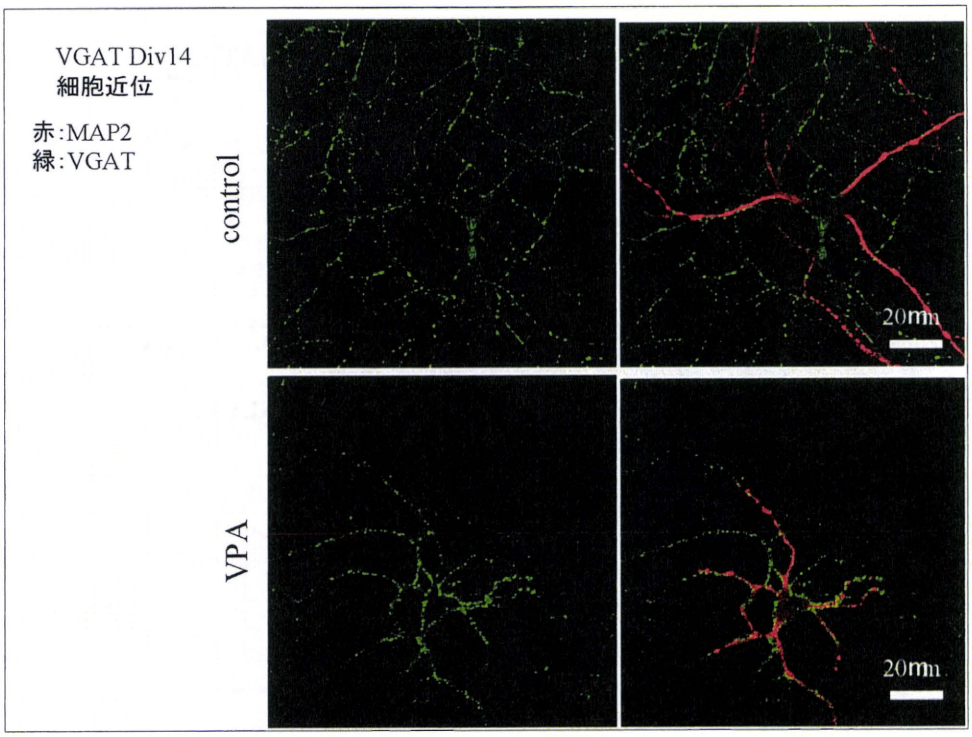
VPA暴露によるVGAT量減少の原因は？

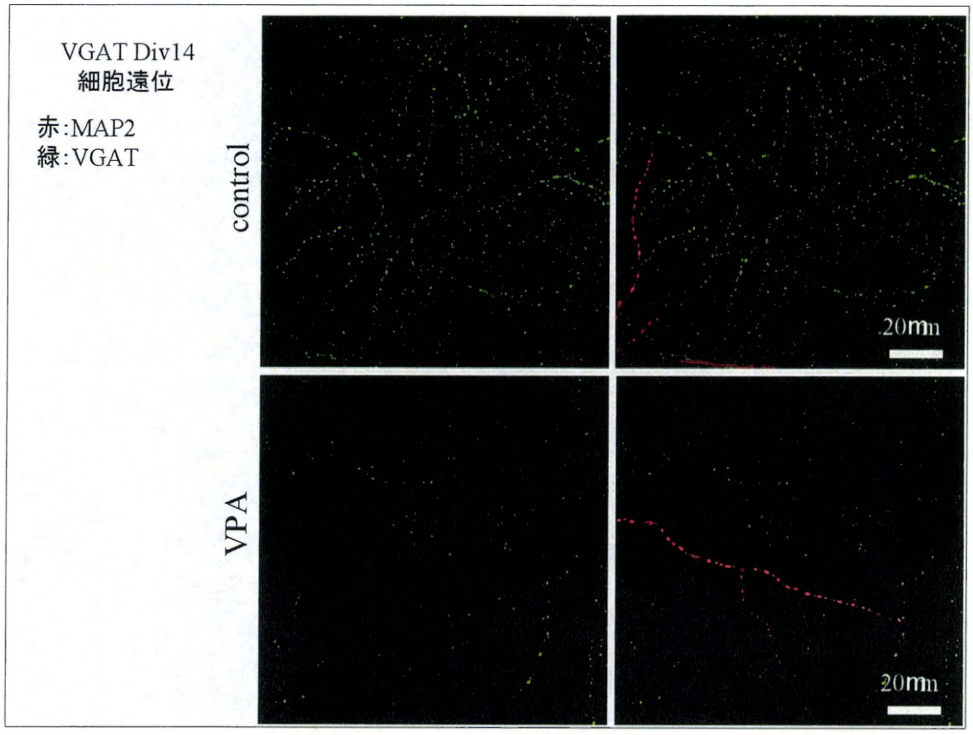
1. VGATの発現量低下

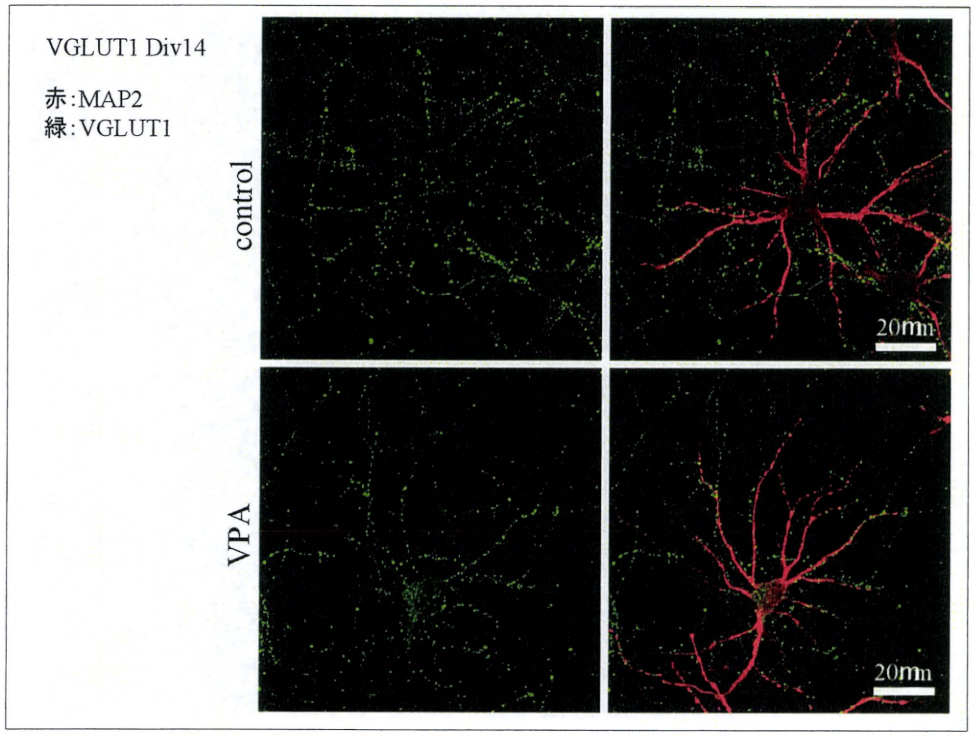
2. GABAシナプスの減少
   - 2a. GABA軸索の発達不全
   - 2b. GABA終末の形成不全

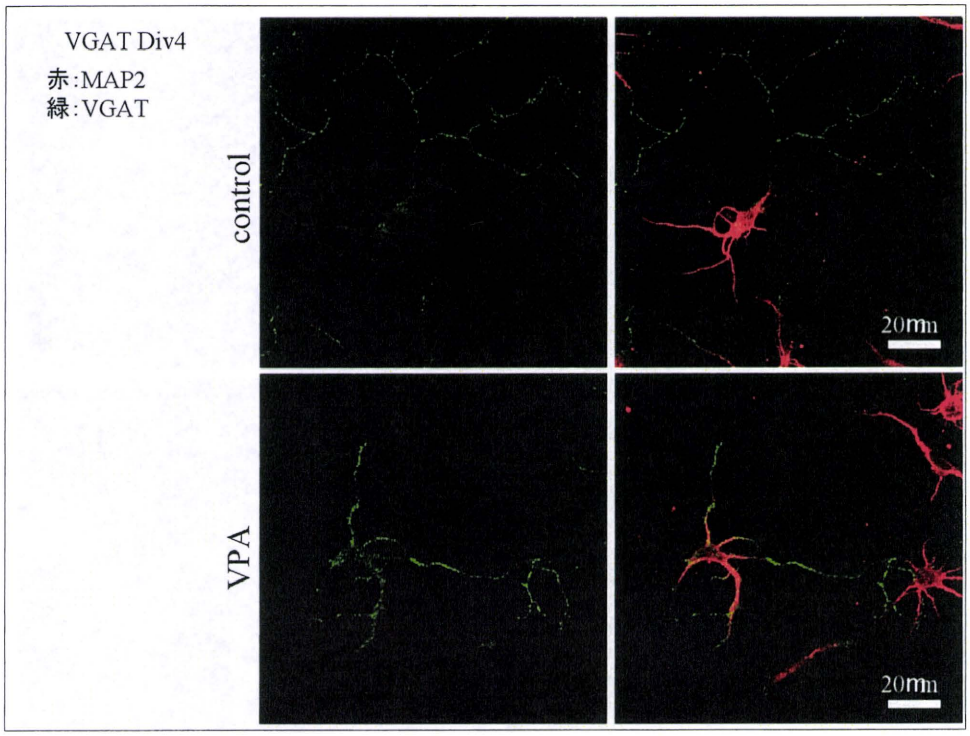
P1由来大脳皮質神経初代培養

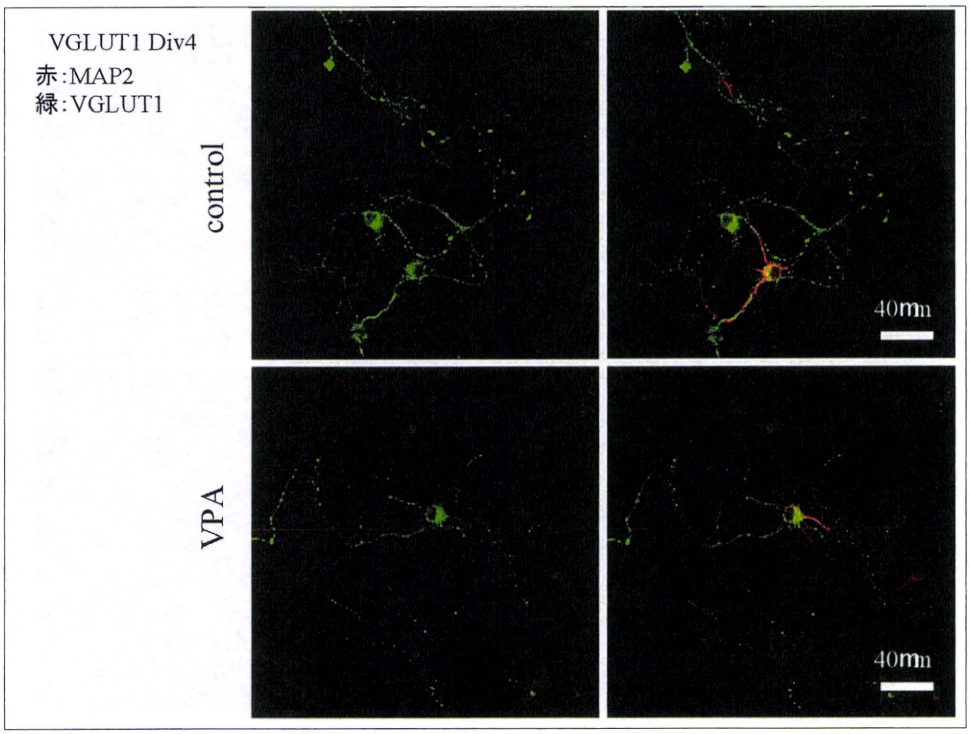


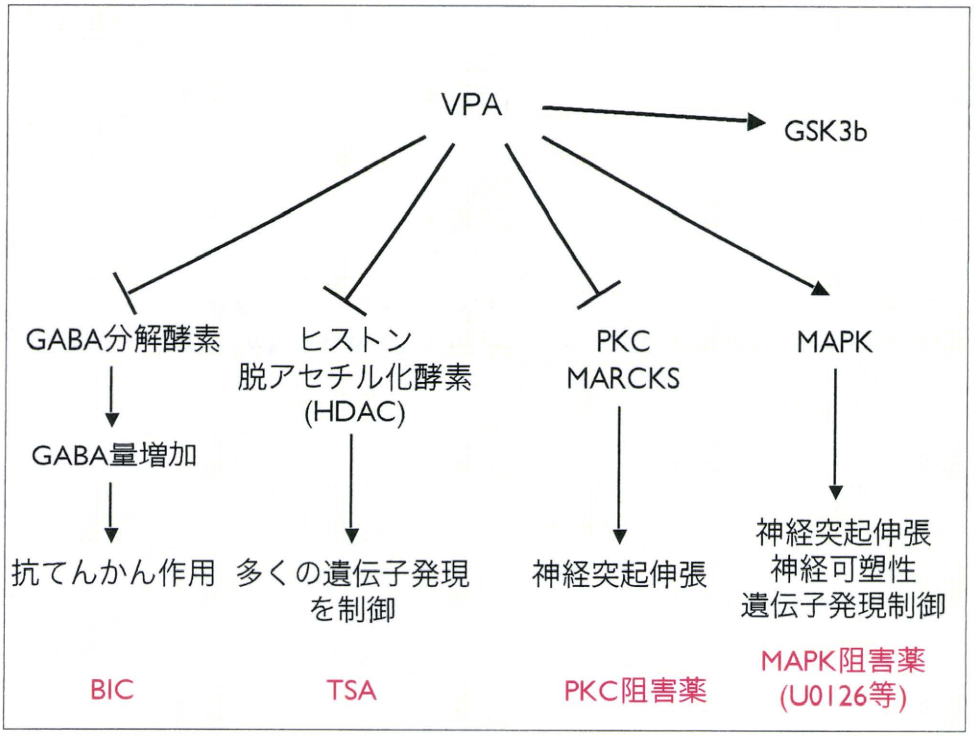


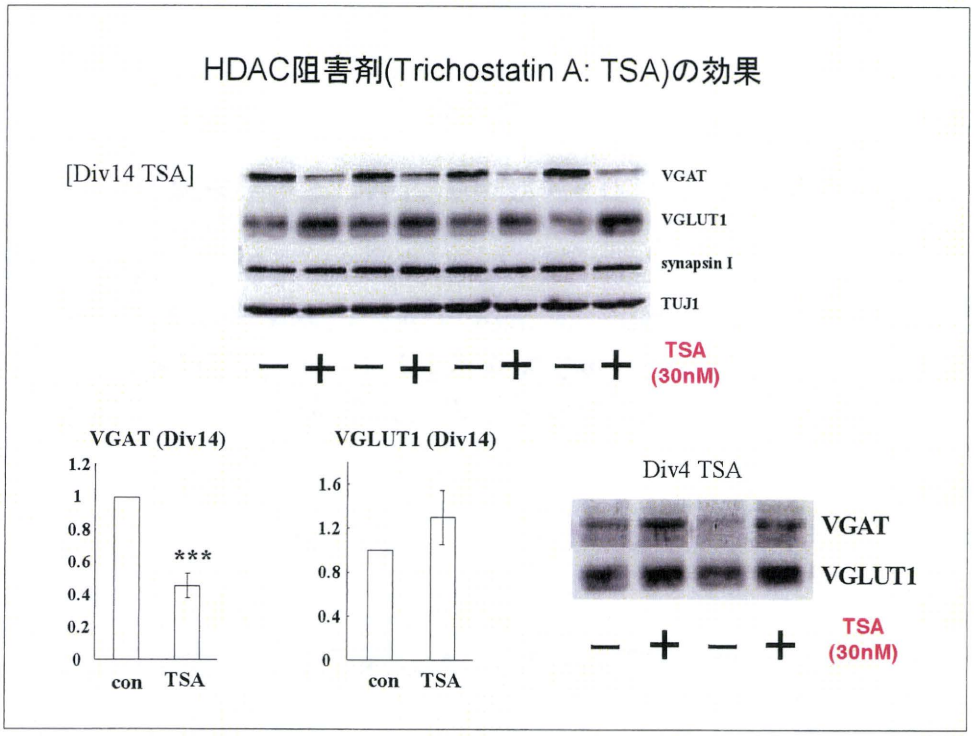

VGAT Div14
細胞遠位
赤:MAP2
緑:VGAT
control
VPA
20mm
20mm


VGLUT1 Div14
赤:MAP2
緑:VGLUT1
control
VPA
20mm
20mm


VGAT Div4
赤:MAP2
緑:VGAT
control
VPA
20mm
20mm


VGLUT1 Div4
赤:MAP2
緑:VGLUT1
control
VPA
40mm
40mm


VPA
GSK3b
GABA分解酵素
GABA量増加
抗てんかん作用
BIC
ヒストン
脱アセチル化酵素
(HDAC)
多くの遺伝子発現
を制御
TSA
PKC
MARCKS
神経突起伸張
PKC阻害薬
MAPK
神経突起伸張
神経可塑性
遺伝子発現制御
MAPK阻害薬
(U0126等)


HDAC阻害剤(Trichostatin A: TSA)の効果
[Div14 TSA]
VGAT
VGLUT1
synapsin I
TUJ1
− + − + − + − +
TSA
(30nM)
VGAT (Div14)
1.2
1
0.8
0.6
0.4
0.2
0
***
con
TSA
VGLUT1 (Div14)
1.6
1.2
0.8
0.4
0
con
TSA
Div4 TSA
VGAT
VGLUT1
− + − +
TSA
(30nM)

Summary

抗てんかん薬として用いられる用量のVPAを妊娠中期の母親マウスに経口投与した場合、産仔マウス海馬におけるE/Iバランスマーカーの顕著な発現変化は見られなかった

大脳皮質由来の幼若神経初代培養細胞を1mM VPAに暴露させると、VGAT発現量の顕著な減少が観察された→E/Iバランスの破綻

VPA暴露によって、抑制性GABA神経の選択的な軸索伸長阻害が観察された。

VPAによるGABA神経特異的な効果は、HDAC阻害活性による可能性が高い。

Outlook

今回の実験では、0, 0.3, 1.0 mM VPAのみ効果を調べた。
Dose–dependencyを精査する。

Axonマーカー(tau–1)の蛍光染色を組み合わせ、dendrite, axon, presynaptic terminalの定量的解析法を確立する

分散培養に加えてオータプス培養を取り入れ、定量解析の簡易化・信頼性の向上を図る。

→ in vitroにおけるE/Iバランス破綻計測のスタンダードな方法を確立する。

→ 興奮性神経と抑制性神経のAxon伸長を司る異なる分子基盤を明らかにする。

別添 5

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
Juliandi B., Abematsu M., Nakashima K.	Epigenetics, Stem cells and Cellular differentiation.	Tollefsbol T.O.	Handbook of epigenetics : The new molecular and medical genetics	Elsevier	オランダ	2010	315 - 328
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雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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S. Watanabe, S. Endo, E. Oshima, H. Higashi, K. Yamada, K. Tohyama, T Yamashita, Y. Hirabayashi	Glycosphingolipid systhesis in cerebellar purkinje neurons: roles in myelin formation and axonal homeostasis.	Glia	58	1197 - 1207	2010
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Mira H., Andreu Z., Suh H., Lie D.C., Jessberger S., Consiglio A., San Emeterio J., Hortiguela R., Marques-Torrejon M.A., Nakashima K., Colak D., Gotz M., Farinas I. & Gage F.H.	Signaling through BMPR-IA regulates quiescence and long-term activity of neural stem cells in the adult hippocampus.	Cell Stem Cell	7	78 - 89	2010

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Oginuma M, Takahashi Y, Kitajima S, Kiso M, Kanno J, Kimura A, Saga Y	The oscillation of Notch activation, but not its boundary, is required for somite border formation and rostral-caudal patterning within a somite.	Developme nt	137 (9)	1515 - 1522	2010
Nogi T, Yasui N, Mihara E, Matsunaga Y, Noda M, Yamashita N, Toyofuku T, Uchiyama S, Goshima Y, Kumanogoh A, Takagi J	Structural basis for semaphorin signalling through the plexin receptor.	Nature	467	1123 - 1127	2010
Viola Maier, Christine Jolicoeur, Helen Rayburn, Noriko Takegahara, Atsushi Kumanogoh, Hitoshi Kikutani, Marc Tessier-Lavigne, Wolfgang Wurst, and Roland H. Friedel	Semaphorin 4C and 4G are ligands of Plexin-B2 required in cerebellar development.	Molecular and Cellular Neuroscien ce			in press
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Yukawa K, Tanaka T, Kishino M, Yoshida K, Takeuchi N, Ito T, Takamatsu H, Kikutani H, Kumanogoh A	Deletion of Sema4D gene reduces intimal neovascularization and plaque growth in apolipoprotein E-deficient mice.	Int J Mol Med	26	39 - 44	2010
Tominaga T, and Tominaga Y	GABA(A) receptor-mediated modulation of neuronal activity propagation upon tetanic stimulation in rat hippocampal slices.	Pflugers Arch	460	875 - 889	2010
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CHAPTER 19

Epigenetics, Stem Cells, and Cellular Differentiation

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[3]Department of Orthopaedic Surgery, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima 890-8520, Japan

INTRODUCTION

Two cardinal features characterize stem cells: their ability to undergo unlimited self-renewal by division and their potential to generate at least two different cell types. Progenitor cells, which possess a limited capacity for self-renewal, are the immediate progeny of stem cells, and behave as transit amplifying cells that can expand the number of new differentiated cells owing to their higher rate of proliferation than the more quiescent stem cells. It can be difficult to distinguish these two cell types unambiguously, and they are sometimes referred to by the collective term "precursor cell".

Numerous studies have indicated that stem cells respond to a combination of intrinsic programs and extracellular cues from the environment that determines which types of progeny they will produce. One of these intrinsic programs is epigenetic modification, which encompasses DNA methylation, chromatin modification, and non-coding RNA-mediated processes. Epigenetic modifications are temporally regulated and reversible, thereby ensuring that stem cells can generate different types of cell from a fixed DNA sequence.

The excitement generated by recent vigorous research on stem cell epigenetic modification reflects the prospect that this new knowledge may enable us to reprogram or modulate the fate of stem cells, using treatments with defined components and at specific time points to alter the epigenetic status of the treated cell and thereby produce a desired cell phenotype. In this review, we discuss recent progress in the study of epigenetic modifications that regulate stem cell differentiation.

STEM CELLS

Animal stem cell research began in the fields of embryology and of the biology of organs with inherent regenerative ability [1]. Other organs with presumptive non-regenerative behavior, such as brain, heart, and lung, were thought to lack stem cells. However, there is increasing evidence that stem cells occur ubiquitously, from embryo to adult and in many organs of the body.

Embryogenesis in multicellular organisms starts with the fertilization of an ovum by a sperm to make a zygote. The zygote is totipotent: it has the potential to develop into a complete

Handbook of Epigenetics: The New Molecular and Medical Genetics. DOI: 10.1016/B978-0-12-375709-8.00019-8

organism and also to make a trophoblast, a structure that will form the placenta. Initial divisions of the zygote yield the morulla and later on the blastocyst, which is composed of the trophoblast, inner cell mass, and blastocyst cavity [2]. The inner cell mass can be isolated and cultured under specific conditions *in vitro* to generate embryonic stem cells (ESCs). ESCs are categorized as pluripotent, since they can generate cells of all body tissues except the trophoblast. This deficiency makes ESCs incapable of forming a complete organism upon implantation into the uterus. Nevertheless, ESCs have the capacity to generate somatic stem cells and subsequently differentiated cells of all three germ layers, ectoderm, mesoderm, and endoderm, if they are pre-treated under optimal *in vitro* culture conditions (Fig. 19.1).

During subsequent developmental stages, each germ layer retains cells that possess stem cell features. These cells are described as being multipotent, because they can generate all progenitor and differentiated cell types within their particular restricted lineage. Neural stem cells (NSCs) and hematopoietic stem cells (HSCs) are examples of such multipotent cells. NSCs can differentiate into neural progenitor cells, neurons, and glial cells (astrocytes and oligodendrocytes). The neuroepithelial cells lining the neural tube are considered as the primary NSCs. From this cell type, the central nervous system develops in a sophisticated temporal and spatial sequence, governed in part by epigenetic mechanisms [3–5]. Likewise, HSCs can give rise to all lineages of the blood, including T and B cells (the lymphoid lineage) and neutrophils, eosinophils, basophils, monocytes, macrophages, megakaryocytes, platelets, and erythrocytes (the myeloid lineage) [6].

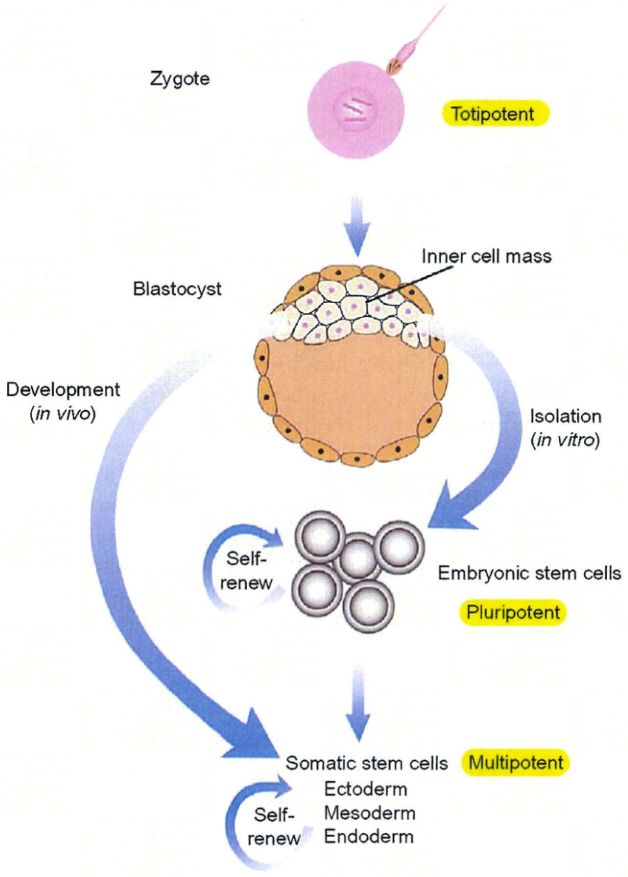

FIGURE 19.1
Developmental potential of stem cells. The totipotent level exists after the egg is fertilized. After several mitotic divisions that lead to the blastocyst, the inner cell mass can be isolated *in vitro*, yielding pluripotent embryonic stem cells (ESCs). ESCs can self-renew and differentiate into multipotent somatic stem cells specific to each of the three germ layers.

EPIGENETICS OF STEM CELLS

There are two pathways in the developmental course of stem cells: either to self-renew while retaining pluripotency or multipotency, or to differentiate into other cell types. During this course, genes that are active at an earlier stage or in maintaining the potency gradually become silenced, and subsets of later-stage genes or cell type-specific genes responsible for the cells' differentiation are then turned on. This reduction in potency over time by progressive gene silencing can be achieved by epigenetic mechanisms in concert with differential expression of certain transcription factors (TFs).

DNA Methylation

Several studies have indicated that DNA methylation regulates the timing of differentiation and maintenance of cell type identity [7–9]. The DNA methylation pattern in the genome is established by a family of DNA methyltransferases (DNMT). Maintenance of methylation patterns is achieved by a function of DNMT1 during DNA replication, while new or *de novo* methylation is primarily catalyzed by DNMT3a and DNMT3b.

In the developing embryonic brain, neurons are generated first and glial cells (astrocytes and oligodendrocytes) afterward. At mid-gestation, embryonic day (E) 11.5 mouse (m) NSCs can only differentiate into neurons, not astrocytes. The glycoprotein 130 and signal transducer and activator of transcription 3 (gp130-STAT3) signaling is a well known pathway to induce astrocytogenesis. Although this pathway can be activated in primary culture of E11.5 mNSCs, its astrocytic target genes are not yet competent to respond to this signal [10,11]. This prevention of premature astrocytogenesis is established in NSCs through methylation in the promoter regions of astrocytic genes, such as glial fibrillary acidic protein (*gfap*). Even in the presence of interleukin-6 (IL-6) family cytokines such as leukemia inhibitory factor (LIF), which can activate the gp130-STAT3 signaling pathway, E11.5 mNSCs do not differentiate into astrocytes because STAT3 cannot bind to the methylated promoter region. The same promoter region becomes hypomethylated as gestation proceeds, which allows the binding of STAT3 and the expression of astrocytic genes in later-stage NSCs [10,12,13], leading them toward astrocytic lineages (Fig. 19.2A).

The astrocyte gene specific demethylation is not just confined to the *gfap* promoter, but is rather common among astrocytic genes. For example, an earlier astrocytic marker *S100β* also possesses a particular cytosine residue in its promoter which is highly methylated in mESCs but becomes lower methylated as mESCs differentiate into neural progenitors [13]. Demethylation of *S100β* promoter also occurs at mid-gestation coinciding with the onset of its expression in the mouse brain [12]. Furthermore, a genome-wide DNA methylation status of E11.5 and E14.5 mNSCs has been recently compared by the profiling method using microarrays [14], confirming that many astrocytic genes become demethylated in late-stage mNSCs.

The *gfap* promoter was also found to be hypomethylated in neurons derived from primary culture of later-stage mNSCs, raising the question of why these cells had not differentiated into astrocytes. Setoguchi et al. [15] showed that even though STAT3 can bind to the hypomethylated *gfap* promoter, *gfap* expression is blocked in neurons due to the association of methyl-CpG-binding protein 2 (MeCP2) with hypermethylated exon 1 (Fig. 19.2A). MeCP2 is a member of the methyl-CpG-binding domain (MBD) proteins, which is highly expressed in neurons. Although recent studies showed that MeCP2 can be found in astrocytes, its expression is very low [16,17]. Indeed, ectopic expression of MeCP2 *in vitro* directs mNSCs to become neurons and inhibits astrocytic differentiation even in the presence of astrocyte-inducing cytokines [18]. Moreover, Tsujimura et al. [18] showed that these astrocytic cytokines actually induce mNSCs to produce more neurons with ectopic MeCP2 expression, by as-yet-unknown mechanisms. It will be intriguing to study how the

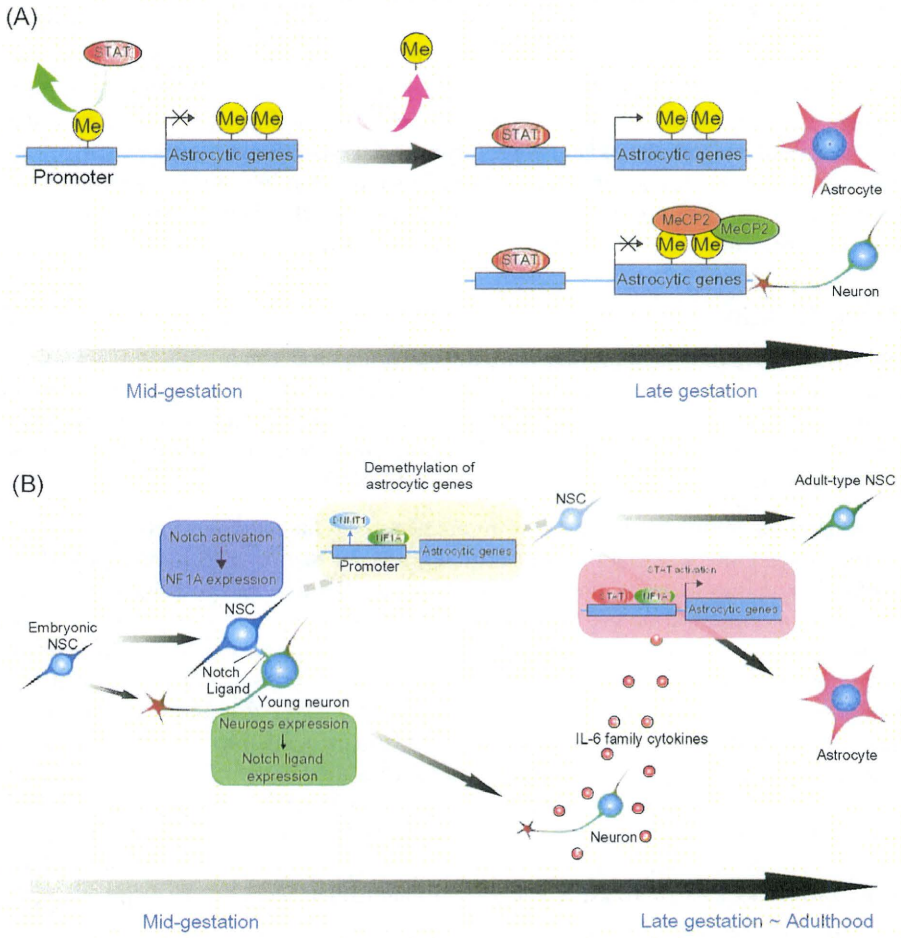

FIGURE 19.2

(A) Astrocytic gene methylation status during NSCs development. Although STAT3 can be activated in mid-gestational NSCs, it cannot bind to astrocytic gene promoters such as *gfap* due to promoter hypermethylation (left). As gestation proceeds, these promoters become demethylated, allowing STAT3 to bind and activate astrocytic genes, resulting in the differentiation of NSCs into astrocytes (upper right). Methyl-CpG binding protein 2 (MeCP2) blocks this activation in neurons (lower right). (B) Notch-induced demethylation of astrocytic genes. Activation of Notch signaling in residual NSCs by young neurons induces demethylation of astrocytic gene promoters by up-regulation of NFIA and release of DNMT1 from astrocytic gene promoters. In turn, at late gestation, IL-6 family cytokines activate the STAT3 pathway and induce NSCs to differentiate into astrocytic lineages.

level of MeCP2 expression and its binding to the astrocytic genes such as *gfap*, influence NSC differentiation in the future. Nevertheless, recent studies show that oligodendrocytes which also have a hypomethylated *gfap* promoter, but unlike neurons which possess MeCP2 at the exon 1 region, can change their fate and become astrocytes by the stimulation of astrocytic cytokines *in vitro* and *in vivo* [17].

A further intriguing question is how astrocytic gene promoters become demethylated in later-stage NSCs. Several reports have indicated that Notch ligands are expressed in neuronally committed precursor cells and young neurons generated from NSCs [19–21]. Notch signaling is a conserved pathway from insects to mammals which contributes to cell-to-cell communication [22–25] and controls cell fate determination in the central nervous system (CNS) [26]. Namihira et al. [11] showed that in the cortex of mouse embryo, Notch ligands are expressed in neuronally committed precursor cells and young neurons, and that Notch signaling is activated in neighboring NSCs (Fig. 19.2B). During this activation, the Notch

intracellular domain (NICD) is released from the plasma membrane and translocated into the nucleus, where it converts the CBF1(RBP-J)/Su(H)/LAG1 repressor complex into an activator complex [27,28]. Forced expression of NICD in primary culture of E11.5 mNSCs induced the up-regulation of nuclear factor IA (NFIA), which in turn accelerated demethylation of astrocytic gene promoters by preventing DNMT1 binding, thus allowing precocious astrocytic differentiation in response to LIF [11] (Fig. 19.2B).

The chicken ovalbumin upstream promoter transcription factors I and II (COUP-TFI/II) were also found to be important for unlocking the silencing epigenetic marks of astrocytic genes [29]. Expression of COUP-TFI/II, which is transiently up-regulated in the early neurogenic period, markedly decreased before the onset of astrocytogenesis. Using mESC-derived NSCs that recapitulate *in vivo* mouse CNS development [30], Naka et al. [29] showed that the CpG methylation status of the *gfap* promoter remained high after COUP-TFI/II knockdown. Moreover, COUP-TFI/II knockdown inhibited the switch from neurogenesis to gliogenesis in this culture system and at developing mouse forebrain. Taken together, these results indicate that COUP-TFI/II are important factors for *gfap* promoter demethylation, although it is not yet clear how demethylation occurs.

The maturation of erythrocytes during hematopoiesis is associated with increased expression of α- and β-globin genes. The β-globin locus consists of five genes, ε, $G\gamma$, $A\gamma$, δ, and β, which are under the regulation of the locus control region located 6–22 kb upstream of the ε-globin gene [31]. In non-erythroid cells, all of these genes are methylated and transcriptionally silent. During erythroid differentiation, individual genes corresponding to embryonic (ε), fetal ($G\gamma$, $A\gamma$), and adult (δ, β) stages of erythropoiesis are expressed in a sequential fashion, such that when the adult genes are activated the embryonic and fetal genes become silenced. Initial activation of embryonic/fetal genes is thought to be caused by demethylation of their promoters, since *in vitro* differentiation of baboon HSCs derived from fetal liver and adult bone marrow into mature erythroblasts is accompanied by a progressive decrease in γ-globin promoter methylation and an activation of transcription [32]. Promoter methylation also occurs in other hematopoietic lineages to regulate differentiation of their precursors. For example, the Ets family transcription factor PU.1 (SPI1) is highly expressed in human (h) HSCs and differentiated B cells, but not in T cells. Hypomethylation of *PU.1* can lead to diffuse large B-cell lymphoma, indicating a requirement for tight DNA methylation control of this gene for normal hematopoiesis [33]. *In vitro* study using murine-derived cell lines showed that differentiation of common progenitors into myeloid and erythroid lineages is also regulated by PU.1 which can recruit SUV39H1, HP1, and retinoblastoma (Rb) proteins when they associate with GATA-1 on its target genes, thereby inhibiting erythroid differentiation [34]. Differential methylation of other genes such as *GATA3*, *TCF-7*, *c-maf*, *TBX21*, and *Etv5* has also been observed to control lineage-commitment in human hematopoiesis [33].

Histone Modification

ACETYLATION

Histone modification processes are at least partially involved in the differentiation of ESCs into NSCs and neurons. mESCs appear to have higher global levels of histone acetylation than lineage-restricted stem cells and differentiated cells, which is consistent with their higher level of transcription and more open chromatin configuration [35]. In fact, heterochromatin markers such as HP1 are highly dynamic and dispersed in the nuclei of mESCs, and then become more concentrated at specific loci as differentiation proceeds [36,37].

Neuron-specific genes are repressed in mESCs by the binding of RE-1 silencing transcription factor (NRSF/REST) to its conserved 21–23 bp DNA response elements (RE-1) which forms a repressor complex by recruiting HDAC1/2 and Sin3A [38–41]. As the cells differentiate into

neural progenitors and neurons, this HDAC containing-repressor complex is released from neuron-specific genes due to the degradation of REST/NRSF [38].

Adult rat (r) hippocampus-derived NSCs differentiate predominantly into neurons, at the expense of astrocytes and oligodendrocytes, if treated by the antiepileptic and HDAC inhibitor valproic acid (VPA) *in vitro*, even in conditions that favor glia-specific differentiation [42]. This HDAC inhibition up-regulates the neuron-specific gene *NeuroD*, a neurogenic basic helix-loop-helix transcription factor, resulting in the induction and suppression of neuronal and glial differentiation, respectively. In the developing rat brain and in cultured E14 rNSCs, VPA treatment can also promote neurogenesis by activating the Ras-ERK pathway [43].

Progression of the oligodendrocyte lineage in rat is also dependent on HDAC activity [44]. Postnatal administration of VPA was shown to delay the timing of NSC differentiation into myelin-forming oligodendrocytes in the developing rat brain [45], and significant hypomyelination in the developing corpus callosum together with sustained expression of progenitor markers and delayed expression of late differentiation markers were observed in this study. However, HDAC inhibition by VPA after the onset of myelination resulted in comparable myelin gene expression with control, attributed to further changes of nucleosomal histones from a state of reversible deacetylation to a more stably repressed state by histone methylation. It has recently been shown that HDAC1/2 contribute to the progression of murine oligodendrocyte differentiation by disrupting the β-catenin-TCF activator complex at inhibitor of differentiation genes *id2/4*, thereby preventing the synthesis of Id2/4 proteins to inhibit myelin gene expression [46].

During hematopoiesis, lineage-restricted TFs can also regulate specific gene expression by recruiting HAT or HDAC complexes to its promoter region [47]. *In vitro* study using erythroid cell line G1E showed that erythroid-specific TFs such as GATA1, which is necessary for red blood cell survival and maturation, recruit HAT-containing complexes to the β-globin locus, inducing acetylation of histones H3 and H4 thus stimulating globin gene expression [48]. Some co-activators such as p300/CBP can also be recruited by TFs to catalyze acetylation of histones, correlating with transcriptional activation of hematopoietic genes [49]. However, p300 can also repress transcription as in the case of acetylation of the promyelocytic leukemia zinc-finger (PLZF) protein [50].

METHYLATION

The methylation of histones on lysine and arginine residues by histone methyl transferase (HMT) represents another level of histone modification. The mixed-lineage leukemia (MLL), which belongs to the trithorax group (trxG) gene, can specifically methylate H3K4 for gene activation by recruiting HATs such as MOF and CBP in various cell lines [51–53], or it can repress target genes through the recruitment of polycomb group (PcG) proteins, HDACs and/or SUV39H1 [54]. In the postnatal mouse brain, MLL1 is required for neurogenesis, and its deficiency in NSCs at the subventricular zone leads to a glial lineage preference [55]. MLL also plays a critical role in the proliferation and lineage determination of hematopoietic progenitors derived from RW4 mESC line, by maintaining the expression of HOX genes, such as *Hoxa7* and *Hoxa9* [56,57], whose up-regulation can confer leukemogenic potential [58].

Stem cell chromatin can be maintained in the bivalent state by PcG proteins [59,60]. The bivalent state is characterized by the existence of both activating and repressive histone methylation marks. In mESCs, while pluripotency-related genes are marked by active histone H3K4 trimethylation (H3K4me3), those that are necessary for differentiation are marked by both activating H3K4me3 and repressive histone H3K27 trimethylation (H3K27me3) chromatin marks [61,62] (Fig. 19.3). Moreover, H3K4me3 is found at nearly 70% of all gene promoters in hESCs [63], while the level of H3K27me3 is only around 10%

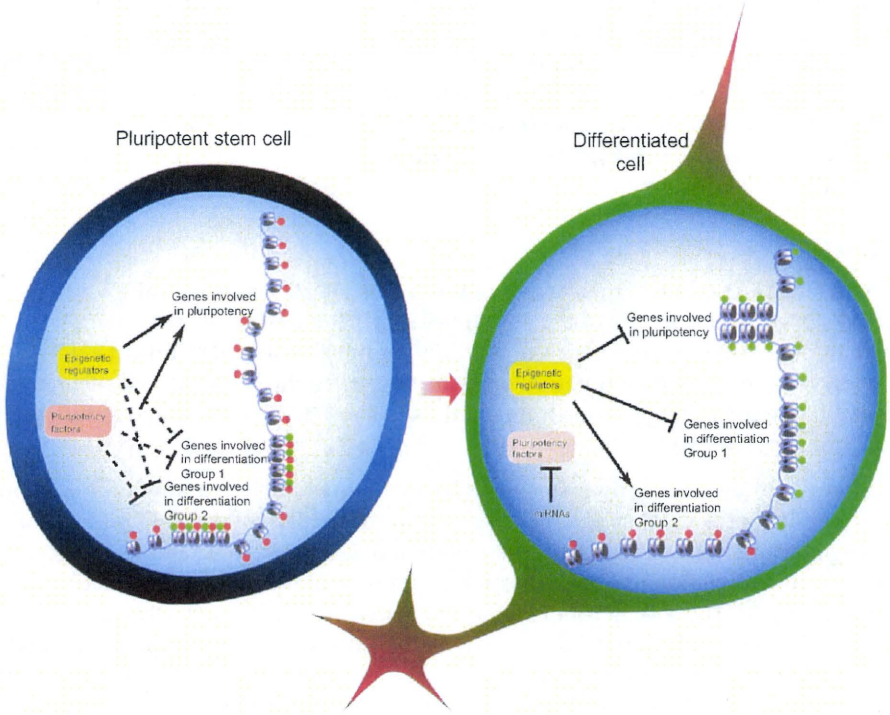

FIGURE 19.3
Regulatory mechanisms of pluripotency. Genes associated with pluripotency are actively transcribed in pluripotent cells (left), while differentiation-associated genes are kept in a silent poised state. Several epigenetic regulators and pluripotency factors regulate this state, in part by a combination of the activating methylation H3K4me3 (red circles) and the repressive methylation H3K27me3 (green circles). In pluripotent cells, only H3K4me3 is present at pluripotency-associated genes, but both H3K4me3 and H3K27me3 at differentiation-associated genes. Upon differentiation, miRNAs down-regulate pluripotency factors and differential repression of differentiation-associated gene groups is sustained only by epigenetic regulators. Pluripotency-associated genes and silenced differentiation-associated gene groups retain the H3K27me3 mark, while activated differentiation-associated gene groups retain the H3K4me3 mark. Chromatin status also changes from hyper to less dynamic during differentiation [36,37].

[59,60,64]. Large blocks of the silent chromatin mark H3K9 methylation also accumulate in differentiated cells rather than in mESCs [65]. In mESC derived-NSCs, on the other hand, the ESC pluripotency-related genes are repressed by methylated H3K9 and the bivalent state exists on neuronal and glial differentiation genes. Thus, it is conceivable that the bivalent state produced by PcG proteins is a common mechanism for maintaining the differentiation potential of many stem/progenitor cell types [64].

Pluripotency is also maintained in human and mouse ESCs by regulatory networks of several TFs, which in some cases, such as Oct3/4, Sox2, and Nanog, are believed to be the main controller [66,67]. Interestingly, these TFs' main direct targets are also transcriptional regulators that might extend the regulatory effects of the network to numerous subsequent targets [68]. Moreover, most of the differentiation regulatory genes are located at the chromatin domains with bivalent state modifications H3K4me3 and H3K27me3 [62] and they are enriched with binding sites for these TFs (Fig. 19.3). This suggests that bivalent-marked differentiation-related genes are kept in a poised state, ready for rapid transcriptional activation upon differentiation under the control of pluripotency TFs, a mechanism that might be responsible for the balance between maintenance of ESC pluripotency and differentiation.

Multipotent stem cells such as NSCs and HSCs, $CD4^+$ T cells and embryonic fibroblasts also possess bivalent histone modification, although less than that in ESCs [64,69–72]. Their lower amount of bivalent histone marks is a result of selective retention: genes whose expression levels are induced upon ESC differentiation retain the H3K4me3 mark, while genes that are silenced keep the H3K27me3 mark [62,64,71] (Fig. 19.3). The persistence of bivalent histone marks at some genes in these cells may ensure the genes' plasticity at later stages of differentiation.

Micro RNA

MicroRNA (miRNA) is one of many types of non-coding RNA, and is typically a 20–25-nucleotide length that can bind to the 3'-untranslated region (UTR) of target mRNAs through an imperfect sequence match to repress their translation and stability [73]. Repression is achieved by the formation of a structure called the RNA-induced silencing complex (RISC). Interestingly, some human miRNAs also have been reported to function as activators of target mRNA translation [74].

Several lines of study using various stem cells have indicated the importance of miRNA in stem cell regulation, and especially in fate specification. The lack of miRNA's maturation processing machinery can resulted in differentiation deficiencies. For example, in Dicer-null mESCs, differentiation marker expression is not present even after the induction of differentiation [75]. The coding regions of pluripotency-markers Nanog, Oct4, and Sox2 in mESCs are also targets of differentiation-related miRNAs such as miR-134, miR-296, and miR-470 [76], and they fail to be silenced in DGCR8-null mESCs [77]. On the other hand, a subset of the miR-290 cluster, called the ES cell-specific cell cycle (ESCC) regulating miRNA, for example miR-291-3p, miR-294, and miR-295 is known to promote proliferation of mESC [78]. Interestingly, these miRNA gene promoters are targets of pluripotency-associated factors Nanog, Oct4, and Sox2 [79]. These observations indicate that the existence of miRNA is both important for ESC differentiation and proliferation, and that miRNAs can promote differentiation by reducing pluripotency-associated protein levels (Fig. 19.3).

In neural tissues, miR-124a is expressed predominantly and has been shown to participate in the *in vitro* differentiation of mNSCs into neurons by mediating degradation of non-neuronal gene transcripts [80]. miR-124a expression is regulated by REST/NRSF, which is expressed only in NSCs and non-neuronal cells including ESCs (see above). In NSCs, therefore, since the expression of the *miR-124a* gene is suppressed by REST/NRSF, the stability of non-neuronal gene transcripts can be increased, thus limiting NSCs to differentiate into neurons. When REST/NRSF is absent, the expression of *miR-124a* and neuronal genes is up-regulated, leading to a preference for neuronal lineage differentiation. miR-124 can also target small carboxy-terminal domain phosphatase 1 (SCP1), which, like REST/NRSF, is an anti-neuronal factor in non-neural tissues and is recruited to RE1-containing gene promoters by REST/NRSF [81], thus providing another mechanism to induce neurogenesis [82]. miR-124a and miR-9 were also found to promote neurogenesis *via* inhibition of STAT3 activation [83]; STAT3 activation induces rNSCs to differentiate into astrocytes while also inhibiting neuronal differentiation [84]. miR-124 and miR-128 are found exclusively in the neuronal lineage, while miR-23, miR-26, and miR-29 are expressed in the astrocytic lineage [85].

Differentiation of early progenitors of the hematopoietic lineage is prevented by miR-128 and miR-181. In addition, another set of miRNAs such as miR-16, miR-103, and miR-107 prevents proliferation of later progenitor cells, whereas miR-221, miR-222, and miR-223 control the terminal differentiation pathways [86]. Mouse hematopoietic progenitor cells can differentiate into lymphoid and myeloid progenitors by selective expression of miR-181 and miR-223, respectively [87]. Within the mouse lymphoid lineage, the differential expression of miR-150 regulates the lineage decision between T- and B-cells [88], while in the myeloid