て働き，神経細胞がグルタミン酸を放出する為に必要不可欠なタンパク質であることを見いだし，VGLUT1（Vesicular glutamate transporter 1）と改名した[5,7]．更に，Edwards のグループは，VGLUT1 タンパク質が Cl-透過性を示す可能性を示した[7]．VGLUT が属する Solute carrier protein（SLC）17 ファミリーのメンバーである SLC17A1 や SLC17A3 に関しても，Cl-の透過性を強く示唆する報告があり[8,9]，Cl-による VGLUT の活性調節機構に対する関心が高まっている（後述）．

VGLUT1 の分子同定当初から，哺乳類脳では幾つかの VGLUT アイソフォームの存在が考えられた．VGLUT1 は大脳皮質・海馬で強く発現しているが，VGLUT1 の発現が認められない視床や間脳部にもグルタミン酸作動性シナプスが存在することは自明だったからである．実際，VGLUT1 に非常に高いアミノ酸相同性を示す遺伝子が次々と同定され，それぞれ VGLUT2, VGLUT3 と命名された．興味深いことに，VGLUT のアイソフォームは，（一部の例外を除いて）脳内で異なるニューロンに発現していることが明らかになっており，今日では部位特異的ノックアウトマウスの開発に基づいた神経回路の生理機能の解明が進み脳神経科学分野の発展に寄与している[10]．

(4) 小胞型グルタミン酸トランスポーターに関わるトランスポートソーム　その存在と分子実体

(4-1) 軸索輸送に関わるトランスポートソーム

VGLUT に限らず，シナプス小胞に局在するタンパク質が選択的に軸索を移動し，最終的に神経終末に到達するメカニズムは，殆ど分かっていないのが現状である．シナプス小胞の膜タンパク質は，細胞体で合成され，ER・ゴルジ装置の経路を経てから軸索を移行する．電子顕微鏡で軸索内をくまなく観察してもシナプス小胞と同等な大きさの小胞構造が見られないことから，シナプス小胞の膜タンパク質は何らかの Precursor 小胞に搭載されて軸索側に運搬されるとする説が有力である．興味深いことに，同じく軸索を通って神経終末に運ばれる Piccolo, Bassoon 等のアクティブゾーンを形成する足場タンパク質群は，直径 80 nm 程の有芯顆粒に搭載されて運ばれているが，その顆粒にはシナプス小胞膜タンパク質は存在しないことが報告された[11]．つまり，シナプス小胞の膜タンパク質はアクティブゾーンタンパク質とは異なる Precursor 小胞によって神経終末に運ばれると考えられる．また，シナプス小胞膜タンパク質の中でも，異なる軸索輸送のモータータンパク質を用いる Precursor 小胞が存在することが示唆されている．このように，個々のシナプス小胞タンパク質がそれぞれの Precursor 小胞によって運搬される過程で，何らかのタンパク質間相互作用やタンパク質-脂質相互作用が関与していることが考えられるが，相互認識を司るシグナル配列は明らかになっておらず，どのような「トランスポートソーム」が関与しているかは，今後の研究課題である．哺乳類の 3 つの VGLUT アイソフォームの内，VGLUT3 のみが神経終末のみならず，細胞体や樹状突起にも存在することは，VGLUT アイソフォームの選択的輸送のメカニズムを解明する上で興味深い[12]．

(4-2) 軸索終末におけるトランスポートソーム

Precursor 小胞は神経終末に到達すると形質膜に融合し，運搬された VGLUT をはじめとした小胞膜タンパク質は，一旦形質膜に挿入される．ここから，エンドサイトーシスによって合成されるシナプス小胞に選択的に挿入されることになる．VGLUT の3つのアイソフォームは，シナプス小胞タンパク質である Synaptotagmin 1 や他の小胞型神経伝達物質トランスポーター (VMAT2, VAChT) と同様，細胞質側に露出した C 末端領域に di-leucine 様のモチーフを有している[13] (図 4-2-6d)．この配列は，AP2 に代表されるアダプタータンパク質に特異的に認識されることで，形質膜から作られるクラスリン被覆小胞への膜タンパク質の挿入を促すといわれている．実際，VGLUT1 タンパク質の C 末端にある FV をアミノ酸置換した変異体は，エキソサイトーシスの後，エンドサイトーシスされる小胞への挿入効率が低く，形質膜に留まる[13]．一方，VGLUT1 のシナプス小胞膜における発現量は，概日リズムと相関して6時間おきに変動することが報告された[14]．この変動は，時計遺伝子である Per2 欠損マウスでは見られず，また他のシナプス小胞タンパク質では見られないことから，VGLUT1 特異的な形質膜—シナプス小胞間の分配機構の存在が想起されるが，その制御機構は不明である．

VGLUT1 特有の輸送機構として知られているのが，エンドサイトーシス関連タンパク質であるエンドフィリンとの直接相互作用である[13]．VGLUT1 の C 末端には di-leucine 様モチーフの他に，Proline-rich domain (PRD) が2つ存在する．この部分をベイトにした Yeast Two Hybrid ス

```
rVGLUT1     EKQPWAEPEEMSEEKCGFVGHDQLAGSDESEMEDEVE   529
rVGLUT2     EKQPWADPEETSEEKCGFIHEDEL--DEETGDITQNY   535
rVGLUT3     EKQDWAKPENLSEEKCGIIDQDELA--EETELNHEAF   539
rVMAT2      PLCFFLRSPPAKEEKMAILMDHNCPIKTKMYTQNNVQ   602
rVAChT      LRNVGLLTRSRRSERDVLLDEPPQGLYDAVRLREVQG   605
rVAMP2      SNRRLQQTQAQVDEVVDIMRVNVDKVLERDKLSELDD    65
mSYT1       RPIAQWHTLQVEEEVDAMLAVKK                 421
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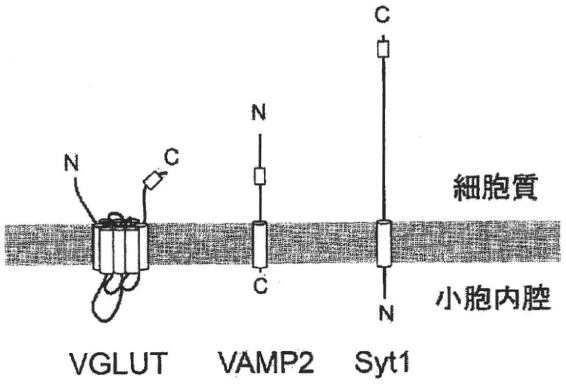

図 4-2-6d　VGLUT の3つアイソフォームは，細胞質側の C 末端部位に di-leucine 様モチーフを持つ（下線）

これらの配列は，他のシナプス小胞膜タンパク質である VAMP2 やシナプトタグミン1 (Syt1) でも見られ，エンドサイトーシスを司るアダプタータンパク質 (AP-2) と直接相互作用することによって，クラスリン被覆小胞への挿入を促す．

クリーニングの結果，Src homology 3 domain（SH3 domain）を持つエンドフィリンが見いだされた．VGLUT2/3 は PRD をもたず，エンドフィリンとの結合能を持たない．エンドフィリン結合能を欠損した VGLUT1 変異体を海馬培養細胞に発現させ，持続的な頻回刺激を与えると，エンドサイトーシスされた小胞に VGLUT1 変異体が取り込まれる速度が低下した．この実験結果は，異なる VGLUT アイソフォームを発現シナプスにおいて，神経活動依存的にグルタミン酸取込速度が異なる可能性を示唆している．

(4-3) 輸送活性に関わるトランスポートソーム

VGLUT のグルタミン酸輸送活性を調節するメカニズムの詳細は明らかになっていない．一方，前述の様に VGLUT は V-ATPase が形成するプロトン電気化学勾配に依存した二次輸送であり，シナプス小胞を用いた研究から，Cl-との何らかの機能的な相互作用が提唱されてきた．本項目では，VGLUT の輸送活性を制御する 2 つの異なるメカニズムについて紹介する．

(4-3-1) Cl-との機能的カップリング

グルタミン酸のシナプス小胞への取込は，小胞外の Cl-濃度に対して二層性の依存性を示すことが古くから知られていた（図 4-2-6b）．また，三量体 G タンパク質の構成成分である $G\alpha O_2$ を欠損したマウスから得られたシナプス小胞を用いた実験から，Cl-の二層性効果には $G\alpha O_2$ の活性化が関与していることが示唆された[15]．しかしながら，それらの分子メカニズムに関しては諸説あり，現在に至っても混沌としている．問題の本質は，Cl-の作用点はどこか？　にある．VGLUT の同定により，VGLUT と輸送の駆動力を供給するプロトンポンプをリポソームに再構

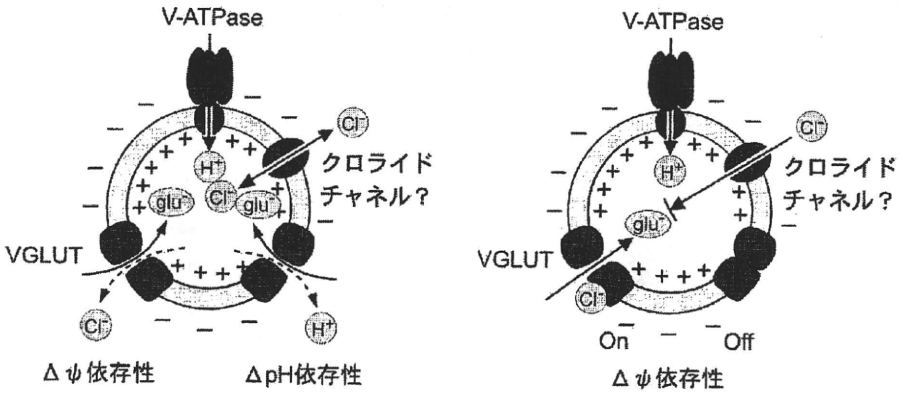

図 4-2-6e　グルタミン酸取込における Cl-依存性の 2 つのモデル

左）VGLUT が膜電位勾配と pH 勾配の双方を駆動力とするモデル．VGLUT が Cl-透過性を有している．小胞外の Cl-は V-ATPase と共役して pH 勾配を作ることにより pH 勾配依存性のグルタミン酸 /H^+交換輸送を行う．それに対して，小胞内に Cl-が存在する場合は，膜電位依存性のグルタミン酸 /Cl-交換体として働く．右）VGLUT が膜電位のみを駆動力とするモデル．この場合，小胞外の Cl-は VGLUT に直接結合し活性化する役割と，分子未同定の Cl-チャネルを通じて流入することで，駆動力である膜電位勾配を低下させる役割の 2 つを担う．

成することが可能になり，筆者らはVGLUT1タンパク質自身がCl-を透過することを見いだした[16]．これは，前述したEdwardsらの実験結果と一致する[7]．興味深いことに，内腔に高濃度のCl-を含むVGLUT1リポソームを作成すると，小胞外にCl-が無い条件においても高い‘膜電位に依存した’グルタミン酸輸送活性が検出された．この実験結果は，VGLUT1がグルタミン酸/Cl-交換体として働きうることを示している[16]．シナプス小胞がエンドサイトーシスにより形質膜から合成される時，小胞内には，高濃度のCl^-を含む細胞外液が流入すると考えられる．従って，筆者らの実験結果は，シナプス小胞内のグルタミン酸量を規定する因子として，細胞外液中のCl-濃度が重要な働きをする可能性を示唆している（図4-2-6e左）．一方で，人工的に膜電位のみ形成させる再構成実験系では，上記の実験結果とは異なり，小胞内のCl-の濃度はグルタミン酸輸送に無関係であることが報告された．この実験で採用された一過性の膜電位形成に依存したグルタミン酸取込測定では，シナプス小胞で見られる高濃度の小胞外Cl^-によるグルタミン酸輸送阻害が見られないことから，Cl-の作用はVGLUTタンパク質への直接結合によるアロステリック効果であると提唱された[17]（図4-2-6e右）．同じリポソームの再構成実験系ではあるが，検出されたグルタミン酸輸送活性は大きく異なっており，シナプス小胞で実際に起きている反応を理解するには，更なる実験系の改良が必要であろう．

(4-3-2) 異種トランスポーターとのシナジー効果

VGLUTの同定により，グルタミン酸作動性神経回路の解剖学的解析が容易になり，哺乳類中枢神経系におけるグルタミン酸放出部位が可視化できるようになった．これら一連の組織化学的解析から，それまで他の神経伝達物質を放出していると考えられていたシナプスの一部でもVGLUTの発現が認められ，グルタミン酸と他の神経伝達物質が共放出されている可能性が示唆された（ドーパミン作動性シナプスにおけるVGLUT2の発現，コリン作動性シナプスにおけるVGLUT3の発現等）．興味深いことに，VGLUT3欠損マウスやドーパミン作動性ニューロン特異

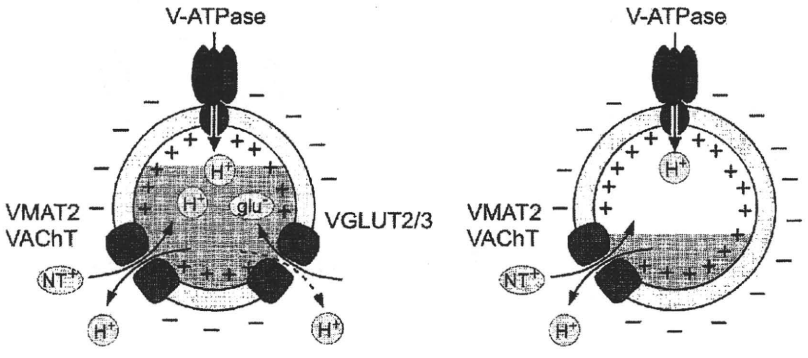

図4-2-6f　異種トランスポーターとのシナジー効果

VGLUTを共発現するモノアミン作動性シナプスやコリン作動性シナプスでは，グルタミン酸取込に伴う小胞内酸性化によってpH勾配が増大し，VGLUTが共発現していないシナプス小胞（右）に比べて，モノアミンやアセチルコリンの取込量が増大する（左）．

的VGLUT2欠損マウスの解析結果から，本来共局在しているVGLUTが欠損すると，ドーパミンやアセチルコリンの小胞内含有量が低下することが分かった[18, 19]．ドーパミンやアセチルコリンは，溶液中で正の電荷を持つ神経伝達物質であり，それらを取り込むトランスポーターであるVMAT2, VAChTは，基質とH^+との交換輸送を司るため，小胞内外のpH勾配を利用して小胞内に取り込まれる．一方，VGLUTを介したグルタミン酸輸送は，小胞内のpHを低下させる．従って，VMAT2やVAChTを含む小胞上にVGLUT（2/3）が存在することでグルタミン酸が取り込まれると，ドーパミンやアセチルコリンの輸送の駆動力であるpH勾配が増大することで，ドーパミン・アセチルコリンの取込量が亢進すると考えられる（図4-2-6f）．この新しい機構は，異種トランスポーター間の「シナジー効果」と呼ばれ，その生理学的意義や脳高次機能への寄与が注目されている．

(5) 展望

VGLUTの分子同定が達成されてから10年余りの間に，グルタミン酸神経回路の神経解剖学的理解が急速に進んだだけでなく，VGLUTタンパク質の輸送体としての構造-機能連関やVGLUTの局在や活性を調節する様々なトランスポートソームの存在が示唆されてきた．今後は，それらトランスポートソームの分子実体の解明や詳細な分子メカニズムの理解が深まっていくだろう．また，トランスポーター自体の作用機序に関してもまだまだ不明な点が多く，*in vitro*の実験結果から導かれたモデルを*in vivo*で再検証する流れの研究が重要になってくると思われる．

（4-2-6　高森茂雄）

参考文献

1) Naito S. & Ueda T. *J. Biol. Chem.* **258**, 696-9, 1983.
2) Naito S. & Ueda T. *J. Neurochem.* **44**, 99-109, 1985.
3) Ni B. *et al. Proc. Natl. Acad. Sci. USA.* **91**, 5607-11, 1994.
4) Bellocchio E.E. *et al. J. Neurosci.* **18**, 8648-59, 1998.
5) Takamori S. *et al. Nature* **407**, 189-94, 2000.
6) Lee R.Y. *et al. J. Neurosci.* **19**, 159-67, 1999.
7) Bellocchio E.E. *et al. Science* **289**, 957-60, 2000.
8) Broer S. *et al. J. Membr. Biol.* **164**, 71-77, 1998.
9) Jutabha P. *et al. J. Biol. Chem.* **285**, 35123-32, 2010.
10) Takamori S. Neurosci. Res. **55**, 343-51, 2006.
11) Shapira M. *et al. Neuron* **38**, 237-52, 2003.
12) Fremeau R.T.Jr. *et al. Proc. Natl. Acad. Sci. USA.* **99**, 14488-93, 2002.
13) Voglmaier S.M. *et al. Neuron* **51**, 71-84, 2006.
14) Yelamanchili S.V. *et al. J. Biol. Chem.* **281**, 15671-9, 2006.
15) Winter S. *et al. J. Neurosci.* **25**, 4672-80, 2005.
16) Schenck S. *et al. Nat. Neurosci.* **12**, 156-62, 2009.
17) Juge N. *et al. Neuron* **68**, 99-112, 2010.

18）Gras C. *et al. Nat. Neurosci.* **11**, 292-300, 2008.
19）Hnasko T.S. *et al. Neuron* **65**, 643-56, 2010.

4-2-7 胃酸分泌細胞のトランスポートソーム

(1) 胃酸分泌に関与するイオン輸送タンパク質

胃酸は，胃粘膜の胃腺に存在する胃酸分泌細胞より分泌される．胃酸分泌細胞は，酸分泌時に劇的な形態変化をおこす．休止時に細胞内に多数存在する細管小胞が，アピカル膜と連結することで，大量の胃酸が分泌される（図4-2-7a）．胃酸分泌細胞は，酸分泌に必要なATPを大量に合成する必要があるため，細胞内に多数のミトコンドリアが存在している．その数はどの上皮細胞よりも多い．

胃プロトンポンプ（H^+, K^+-ATPase）は，サブユニット構造をとり，触媒サブユニット（α鎖）と非触媒サブユニット（β鎖）からなる．P型ATPaseファミリーに属し，ATPの加水分解の際に生成する無機リン酸を直接結合した中間体を一時的に形成する．H^+, K^+-ATPaseは，細胞膜を隔てた100万倍以上ものプロトン濃度勾配に逆らってH^+を胃管腔（pHは約1）へ分泌する．我々は最近，H^+, K^+-ATPaseのプロトン輸送に関与する電荷移動路を明らかにした[1]．

胃酸（HCl）のH^+輸送がH^+, K^+-ATPaseにより行われていることはよく知られている一方で，Cl^-輸送を担う分子については種々議論されている．これまでにCFTR[2]，SLC26A9[3]，CLIC-6[4]などのCl^-チャネルが候補として報告されている．また，H^+, K^+-ATPaseが活性化されH^+の分泌を維持するには，細胞内へのK^+の安定な供給が必要不可欠である．これまでに管腔側膜におけるこのK^+供給実体について，KCNQ1/KCNE2[5]やKir4.1[6]などのK^+チャネルが報告されている．

K^+-Cl^-共輸送体（KCC）は，K^+とCl^-を細胞外に共輸送する二次性能動輸送体であり，種々の上皮細胞において細胞内Cl^-濃度調節や細胞容積調節などに関与している．KCCにはこれま

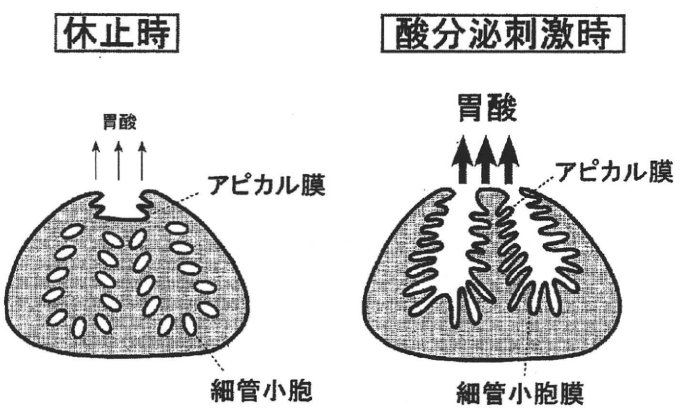

図4-2-7a　休止時および酸分泌刺激時の胃酸分泌細胞の形態

CHAPTER 19

Epigenetics, Stem Cells, and Cellular Differentiation

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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

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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].

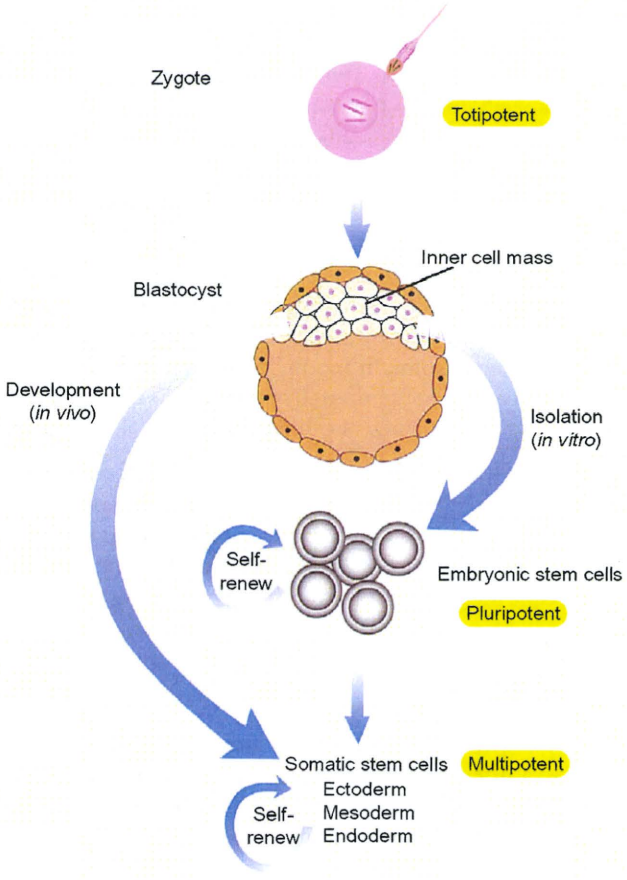

FIGURE 19.1
Developmental potential of stem cells. The totipotent level exists after the egg is fertilized. After several mitotic divisions that lead to the blastocyst, the inner cell mass can be isolated *in vitro*, yielding pluripotent embryonic stem cells (ESCs). ESCs can self-renew and differentiate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

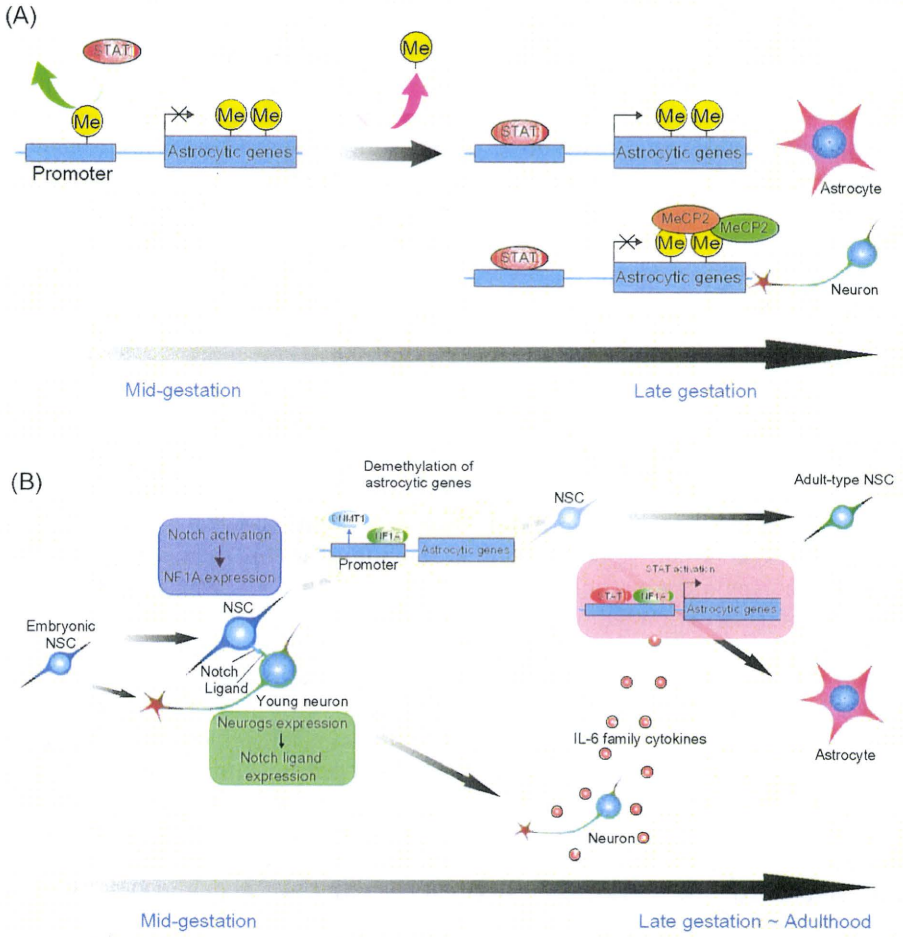

FIGURE 19.2
(A) Astrocytic gene methylation status during NSCs development. Although STAT3 can be activated in mid-gestational NSCs, it cannot bind to astrocytic gene promoters such as *gfap* due to promoter hypermethylation (left). As gestation proceeds, these promoters become demethylated, allowing 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%

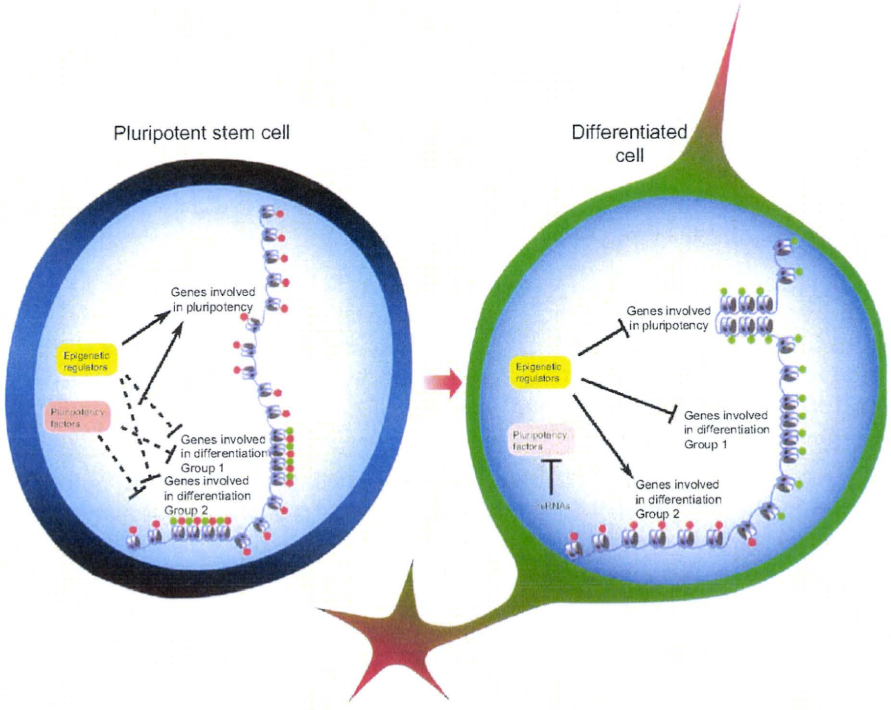

FIGURE 19.3
Regulatory mechanisms of pluripotency. Genes associated with pluripotency are actively transcribed in pluripotent cells (left), while differentiation-associated genes are kept in a silent poised state. Several epigenetic regulators and pluripotency factors regulate this state, in part by 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

lineage miR-150, miR-155, miR-221, and miR-222 are progressively down-regulated, with up-regulation of miR-451 and miR-16 occurring during the late phase of human erythropoiesis in *in vitro* study [89].

REPROGRAMMING FOR PLURIPOTENCY

Cell differentiation has been depicted as a ball rolling down an epigenetic landscape [90], starting from totipotency, moving through pluripotency, and finally reaching lineage-committed states. In the last three years, multiple studies have reported that the "ball" can actually be pushed back up the hill (Fig. 19.4): several types of differentiated cells have been shown to be reprogrammable back to the pluripotent state under the influence of a few factors such as Oct4, Sox2, Klf4, Myc, Nanog, and Lin-28 [91–101]. Such reprogrammed cells, which have been called induced pluripotent stem (iPS) cells [91], are similar to ESCs in terms of their morphology, expression of major ESC marker genes, and capacity to self-renew and to differentiate into various cell types of the three germ layers.

Induced pluripotency in iPS cells was shown to be caused by changes in epigenetic modification of the treated cells. The promoter regions of various pluripotency-associated genes are hypermethylated in differentiated cells, but in iPS cells these genes are hypomethylated, resembling their state in ESCs [102]. How the above-mentioned inducing factors can trigger the demethylation of pluripotency genes remains elusive, because it is unclear whether they possess direct or indirect DNA demethylation activity. Interestingly, nevertheless, generation of iPS cells can be promoted by demethylating agents such as 5-azacytidine [103], underlining the importance of the DNA demethylation process in mediating induced pluripotency.

Bivalent methylation marks on histone H3 are also re-established at the promoter regions in iPS cells [103,104]. Both ESCs and iPS cells have H3K4me3 in the promoter regions of pluripotency-associated genes, while both active H3K4me3 and repressive H3K27me3 are present at their differentiation-associated genes [62] (Fig. 19.3). Because differentiated cells such as mouse fibroblasts have the opposite histone methylation pattern [104], it is very likely that histone methylation also plays a role in reprogramming.

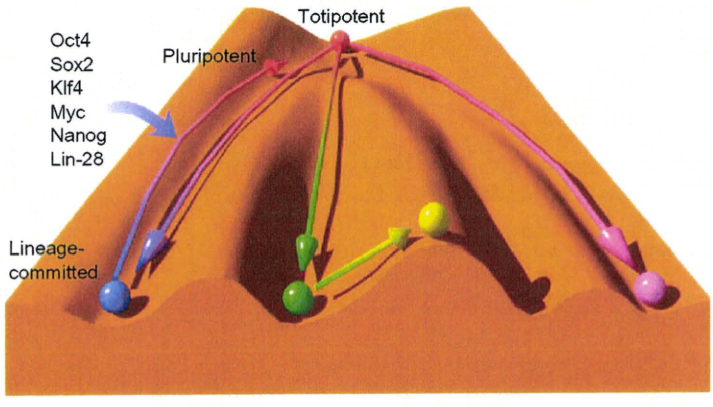

FIGURE 19.4
Epigenetic landscape. The totipotent fertilized egg can be depicted as a red ball that can roll down one of several possible valleys, passing through the pluripotent state and then differentiating into a particular tissue-lineage cell (blue, green, and pink balls). Reprogramming factors can push the ball back up the hill, enabling it to re-acquire pluripotency features. Lineage-committed cells can also trans-differentiate into cells of another lineage (yellow ball) by other epigenetic reprogrammings. The diagram is modified from Waddington [90].

In both ESCs and iPS cells, histones H3 and H4 at the promoter regions of pluripotency-associated genes are hyperacetylated, whereas these promoters in differentiated cells display hypoacetylated H3 and H4. Addition of the HDAC inhibitor VPA can lead to a hyperacetylated histone status, and has proved to be efficient in inducing pluripotency of human fibroblasts, with only Oct4 and Sox2 required as necessary supplemental factors [105]. Other factors may be dispensable under these conditions if any role they play in increasing acetylation by recruiting HATs, a known function in the case of c-Myc [106], can be substituted by VPA.

CONCLUDING REMARKS

Revealing the epigenetic mechanisms that contribute to stem cell potency and differentiation has been an exciting journey. Still, many avenues remain to be explored. For example, several HDAC inhibitors and DNA demethylating agents such as VPA and 5-azacytidine are now in clinical trials for therapeutic application to several disorders and diseases [107,108]. While HDAC inhibitors or DNA demethylating agents might be expected to affect a broad range of genes or their activator/repressor complexes, they actually do not. This differential effect must be attributable to characteristics that the affected genes alone possess. Therefore, the knowledge of how these compounds relieve or cure disorder and disease by changing epigenetic marks is highly important. It will be also interesting to explore the effect of disruption of certain miRNAs in order to generate iPS cells, because recent findings show that RNA binding protein Lin28 can promote reprogramming by selective inhibition of miRNA maturation machinery [109,110]. We also have to evaluate carefully the various origins of cells and induction methods available for stem cell differentiation in order to generate specific cell phenotypes that can be used for clinical applications. To develop an optimal method to generate specific cell types, we must urgently learn more about the precise mechanisms of stem cell fate specification.

ACKNOWLEDGEMENTS

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References

1. Kempermann G. Adult neurogenesis. New York: Oxford University Press; 2006.
2. Keller G. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev* 2005;**19**:1129–55.
3. Namihira M, Kohyama J, Abematsu M, Nakashima K. Epigenetic mechanisms regulating fate specification of neural stem cells. *Phil Trans R Soc B* 2008;**363**:2099–109.
4. Allen ND. Temporal and epigenetic regulation of neurodevelopmental plasticity. *Phil Trans R Soc B* 2008;**363**:23–8.
5. Okano H, Temple S. Cell types to order: temporal specification of CNS stem cells. *Curr Opin Neurobiol* 2009;**19**:1–8.
6. Zhu J, Emerson SG. Hematopoietic cytokines, transcription factors and lineage commitment. *Oncogene* 2002;**21**:3295–313.
7. Liang G, Chan MF, Tomigahara Y, Tsai YC, Gonzales FA, Li E, et al. Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. *Mol Cell Biol* 2002;**22**:480–91.
8. Shiota K, Kogo Y, Ohgane J, Imamura T, Urano A, Nishino K, et al. Epigenetic marks by DNA methylation specific to stem, germ and somatic cells in mice. *Genes Cells* 2002;**7**:961–9.

9. Bibikova M, Chudin E, Wu B, Zhou L, Garcia EW, Liu Y, et al. Human embryonic stem cells have a unique epigenetic signature. *Genome Res* 2006;**16**:1075–83.
10. Takizawa T, Nakashima K, Namihira M, Ochiai W, Uemura A, Yanagisawa M, et al. DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. *Dev Cell* 2001;**1**:749–58.
11. Namihira M, Kohyama J, Semi K, Sanosaka T, Deneen B, Taga T, et al. Committed neuronal precursors confer astrocytic potential on residual neural precursor cells. *Dev Cell* 2009;**16**:245–55.
12. Namihira M, Nakashima K, Taga T. Developmental stage dependent regulation of DNA methylation and chromatin modification in a immature astrocyte specific gene promoter. *FEBS Lett* 2004;**572**:184–8.
13. Shimozaki K, Namihira M, Nakashima K, Taga T. Stage- and site-specific DNA demethylation during neural cell development from embryonic stem cells. *J Neurochem* 2005;**93**:432–9.
14. Hatada I, Namihira M, Morita S, Kimura M, Horii T, Nakashima K. Astrocyte-specific genes are generally demethylated in neural precursor cells prior to astrocytic differentiation. *PLoS One* 2008;**3**:e3189.
15. Setoguchi H, Namihira M, Kohyama J, Asano H, Sanosaka T, Nakashima K. Methyl-CpG binding proteins are involved in restricting differentiation plasticity in neurons. *J Neurosci Res* 2006;**84**:969–79.
16. Ballas N, Lioy DT, Grunseich C, Mandel G. Non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. *Nat Neurosci* 2009;**12**:311–17.
17. Kohyama J, Kojima T, Takatsuka E, Yamashita T, Namiki J, Hsieh J, et al. Epigenetic regulation of neural cell differentiation plasticity in the adult mammalian brain. *Proc Natl Acad Sci USA* 2008;**105**:18012–17.
18. Tsujimura K, Abematsu M, Kohyama J, Namihira M, Nakashima K. Neuronal differentiation of neural precursor cells is promoted by the methyl-CpG binding protein MeCP2. *Exp Neurol* 2009;**219**:104–11.
19. Campos LS, Duarte AJ, Branco T, Henrique D. mDll1 and mDll3 expression in the developing mouse brain: role in the establishment of the early cortex. *J Neurosci Res* 2001;**64**:590–8.
20. Kawaguchi A, Ikawa T, Kasukawa T, Ueda HR, Kurimoto K, Saitou M, et al. Single-cell gene profiling defines differential progenitor subclasses in mammalian neurogenesis. *Development* 2008;**135**:3113–24.
21. Yoon KJ, Koo BK, Im SK, Jeong HW, Ghim J, Kwon MC, et al. Mind bomb 1-expressing intermediate progenitors generate notch signaling to maintain radial glial cells. *Neuron* 2008;**58**:519–31.
22. Simpson P. Developmental genetics. The Notch connection. *Nature* 1995;**375**:736–7.
23. Nye JS, Kopan R. Developmental signaling. Vertebrate ligands for Notch. *Curr Biol* 1995;**5**:966–9.
24. Bray S. A Notch affair. *Cell* 1998;**93**:499–503.
25. Louvi A, Artavanis-Tsakonas S. Notch signaling in vertebrate neural development. *Nat Rev Neurosci* 2006;**7**:93–102.
26. Lundkvist J, Lendahl U. Notch and the birth of glial cells. *Trends Neurosci* 2001;**24**:492–4.
27. Nakayama K, Nagase H, Hiratochi M, Koh CS, Ohkawara T. Similar mechanisms regulated by gamma-secretase are involved in both directions of the bi-directional Notch-Delta signaling pathway as well as play a potential role in signaling events involving type 1 transmembrane proteins. *Curr Stem Cell Res Ther* 2008;**3**:288–302.
28. Wallberg AE, Pedersen K, Lendahl U, Roeder RG. p300 and PCAF act cooperatively to mediate transcriptional activation from chromatin templates by notch intracellular domains in vitro. *Mol Cell Biol* 2002;**22**:7812–19.
29. Naka H, Nakamura S, Shimazaki T, Okano H. Requirement for COUP-TFI and II in the temporal specification of neural stem cells in central nervous system development. *Nat Neurosci* 2008;**11**:1014–23.
30. Okada Y, Matsumoto A, Shimazaki T, Enoki R, Koizumi A, Ishii S, et al. Spatio-temporal recapitulation of central nervous system development by murine ES cell-derived neural stem/progenitor cells. *Stem Cells* 2008;**26**:3086–98.
31. Levings PP, Bungert J. The human beta-globin locus control region. *Eur J Biochem* 2002;**269**:1589–99.
32. Singh M, Lavelle D, Vaitkus K, Mahmud N, Hankewych M, DeSimone J. The gamma-globin gene promoter progressively demethylates as the hematopoietic stem progenitor cells differentiate along the erythroid lineage in baboon fetal liver and adult bone marrow. *Exp Hematol* 2007;**35**:48–55.
33. Ivascu C, Wasserkort R, Lesche R, Dong J, Stein H, Thiel A, et al. DNA methylation profiling of transcription factor genes in normal lymphocyte development and lymphomas. *Int J Biochem Cell Biol* 2007;**39**:1523–38.
34. Stopka T, Amanatullah DF, Papetti M, Skoultchi AI. PU.1 inhibits the erythroid program by binding to GATA-1 on DNA and creating a repressive chromatin structure. *EMBO J* 2005;**24**:3712–23.
35. Efroni S, Duttagupta R, Cheng J, Dehghani H, Hoeppner DJ, Dash C, et al. Global transcription in pluripotent embryonic stem cells. *Cell Stem Cell* 2008;**2**:437–47.
36. Meshorer E, Yellajoshula D, George E, Scambler PJ, Brown DT, Misteli T. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. *Dev Cell* 2006;**10**:105–16.
37. Meshorer E, Misteli T. Chromatin in pluripotent embryonic stem cells and differentiation. *Nat Rev Mol Cell Biol* 2006;**7**:540–6.
38. Ballas N, Grunseich C, Lu DD, Speh JC, Mandel G. REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell* 2005;**121**:645–57.

39. Lunyak VV, Burgess R, Prefontaine GG, Nelson C, Sze SH, Chenoweth J, et al. Co-repressor-dependent silencing of chromosomal regions encoding neuronal genes. *Science* 2002;**298**:1747–52.

40. Lunyak VV, Rosenfeld MG. No rest for REST: REST/NRSF regulation of neurogenesis. *Cell* 2005;**121**:499–501.

41. Rice JC, Allis CD. Histone methylation versus histone acetylation: new insights into epigenetic regulation. *Curr Opin Cell Biol* 2001;**13**:263–73.

42. Hsieh J, Nakashima K, Kuwabara T, Mejia E, Gage FH. Histone deacetylase inhibition-mediated neuronal differentiation of multipotent adult neural progenitor cells. *Proc Natl Acad Sci USA* 2004;**101**:16659–64.

43. Jung GA, Yoon JY, Moon BS, Yang DH, Kim HY, Lee SH, et al. Valproic acid induces differentiation and inhibition of proliferation in neural progenitor cells via the beta-catenin-Ras-ERK-p21$^{Cip/WAF1}$ pathway. *BMC Cell Biol* 2008;9:66.

44. Marin-Husstege M, Muggironi M, Liu A, Casaccia-Bonnefil P. Histone deacetylase activity is necessary for oligodendrocyte lineage progression. *J Neurosci* 2002;**22**:10333–45.

45. Shen S, Li J, Casaccia-Bonnefil P. Histone modifications affect timing of oligodendrocyte progenitor differentiation in the developing rat brain. *J Cell Biol* 2005;**169**:577–89.

46. Ye F, Chen Y, Hoang TN, Montgomery RL, Zhao X, Bu H, et al. HDAC1 and HDAC2 regulate oligodendrocyte differentiation by disrupting the β-catenin-TCF interaction. *Nat Neurosci* 2009;**12**:829–38.

47. Huo X, Zhang J. Important roles of reversible acetylation in the function of hematopoietic transcription factors. *J Cell Mol Med* 2005;**9**:103–12.

48. Letting DL, Rakowski C, Weiss MJ, Blobel GA. Formation of a tissue-specific histone acetylation pattern by the hematopoietic transcription factor GATA-1. *Mol Cell Biol* 2003;**23**:1334–40.

49. Blobel GA. CBP and p300: versatile coregulators with important roles in hematopoietic gene expression. *J Leukoc Biol* 2002;**71**:545–56.

50. Guidez F, Howell L, Isalan M, Cebrat M, Alani RM, Ivins S, et al. Histone acetyltransferase activity of p300 is required for transcriptional repression by the promyelocytic leukemia zinc finger protein. *Mol Cell Biol* 2005;**25**:5552–66.

51. Ernst P, Wang J, Huang M, Goodman RH, Korsmeyer SJ. MLL and CREB bind cooperatively to the nuclear coactivator CREB-binding protein. *Mol Cell Biol* 2001;**21**:2249–58.

52. Milne TA, Briggs SD, Brock HW, Martin ME, Gibbs D, Allis CD, et al. MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol Cell* 2002;**10**:1107–17.

53. Dou Y, Milne TA, Tackett AJ, Smith ER, Fukuda A, Wysocka J, et al. Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. *Cell* 2005;**121**:873–85.

54. Xia ZB, Anderson M, Diaz MO, Zeleznik-Le NJ. MLL repression domain interacts with histone deacetylases, the polycomb group proteins HPC2 and BMI-1, and the corepressor C-terminal-binding protein. *Proc Natl Acad Sci USA* 2003;**100**:8342–7.

55. Lim DA, Huang YC, Swigut T, Mirick AL, Garcia-Verdugo JM, Wysocka J, et al. Chromatin remodelling factor Mll1 is essential for neurogenesis from postnatal neural stem cells. *Nature* 2009;**458**:529–33.

56. Ernst P, Wang J, Korsmeyer SJ. The role of MLL in hematopoiesis and leukemia. *Curr Opin Hematol* 2002;9:282–7.

57. Ernst P, Mabon M, Davidson AJ, Zon LI, Korsmeyer SJ. An Mll-dependent Hox program drives hematopoietic progenitor expansion. *Curr Biol* 2004;**14**:2063–9.

58. Ayton PM, Cleary ML. Transformation of myeloid progenitors by MLL oncoproteins is dependent on Hoxa7 and Hoxa9. *Genes Dev* 2003;**17**:2298–307.

59. Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* 2006;**441**:349–53.

60. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, et al. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* 2006;**125**:301–13.

61. Azuara V, Perry P, Sauer S, Spivakov M, Jørgensen HF, John RM, et al. Chromatin signatures of pluripotent cell lines. *Nat Cell Biol* 2006;**8**:532–8.

62. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 2006;**125**:315–26.

63. Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA. A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* 2007;**130**:77–88.

64. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 2007;**448**:553–60.

65. Wen B, Wu H, Shinkai Y, Irizarry RA, Feinberg AP. Large histone H3 lysine 9 dimethylated chromatin blocks distinguish differentiated from embryonic stem cells. *Nat Genet* 2009;**41**:246–50.

66. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 2005;**122**:947–56.

67. Loh YH, Wu Q, Chew JL, Vega VB, Zhang W, Chen X, et al. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nat Genet* 2006;**38**:431–40.

68. Zhou Q, Chipperfield H, Melton DA, Wong WH. A gene regulatory network in mouse embryonic stem cells. *Proc Natl Acad Sci USA* 2007;**104**:16438–43.

69. Roh TY, Cuddapah S, Cui K, Zhao K. The genomic landscape of histone modifications in human T cells. *Proc Natl Acad Sci USA* 2006;**103**:15782–7.

70. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, et al. High-resolution profiling of histone methylations in the human genome. *Cell* 2007;**129**:823–37.

71. Pan G, Tian S, Nie J, Yang C, Ruotti V, Wei H, et al. Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. *Cell Stem Cell* 2007;**1**:299–312.

72. Cui K, Zang C, Roh TY, Schones DE, Childs RW, Peng W, et al. Chromatin signatures in multipotent human hematopoeitic stem cells indicate the fate of bivalent genes during differentiation. *Cell Stem Cell* 2009;**4**:80–93.

73. Rana TM. Illuminating the silence: understanding the structure and function of small RNAs. *Nat Rev Mol Cell Biol* 2007;**8**:23–36.

74. Vasudevan S, Tong Y, Steitz JA. A switching from repression to activation: microRNAs can up-regulate translation. *Science* 2007;**318**:1931–4.

75. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, et al. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev* 2005;**19**:489–501.

76. Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I. MicroRNAs to Nanog, Oct4, and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature* 2008;**455**:1124–8.

77. Wang Y, Medvid R, Melton C, Jaenisch R, Blelloch R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nat Genet* 2007;**39**:380–5.

78. Wang Y, Baskerville S, Shenoy A, Babiarz JE, Baehner L, Blelloch R. Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. *Nat Genet* 2008;**40**:1478–83.

79. Marson A, Levine SS, Cole MF, Frampton GM, Brambrink T, Johnstone S, et al. Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. *Cell* 2008;**134**:521–33.

80. Conaco C, Otto S, Han JJ, Mandel G. Reciprocal actions of REST and a microRNA promote neuronal identity. *Proc Natl Acad Sci USA* 2006;**103**:2422–7.

81. Yeo M, Lee SK, Lee B, Ruiz EC, Pfaff SL, Gill GN. Small CTD phosphatases function in silencing neuronal gene expression. *Science* 2005;**307**:596–600.

82. Visvanathan J, Lee S, Lee B, Lee JW, Lee SK. The microRNA miR-124 antagonizes the anti-neural REST/SCP1 pathway during embryonic CNS development. *Genes Dev* 2007;**21**:744–9.

83. Krichevsky AM, Sonntag KC, Isacson O, Kosik KS. Specific microRNAs modulate embryonic stem cell-derived neurogenesis. *Stem Cells* 2006;**24**:857–64.

84. Gu F, Hata R, Ma YJ, Tanaka J, Mitsuda N, Kumon Y, et al. Suppression of Stat3 promotes neurogenesis in cultured neural stem cells. *J Neurosci Res* 2005;**81**:163–71.

85. Smirnova L, Gräfe A, Seiler A, Schumacher S, Nitsch R, Wulczyn FG. Regulation of miRNA expression during neural cell specification. *Eur J Neurosci* 2005;**21**:1469–77.

86. Georgantas RW 3rd, Hildreth R, Morisot S, Alder J, Liu CG, Heimfeld S, et al. CD34+ hematopoietic stem-progenitor cell microRNA expression and function: a circuit diagram of differentiation control. *Proc Natl Acad Sci USA* 2007;**104**:2750–5.

87. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science* 2004;**303**:83–6.

88. Zhou B, Wang S, Mayr C, Bartel DP, Lodish HF. miRNA-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely. *Proc Natl Acad Sci USA* 2007;**104**:7080–5.

89. Bruchova H, Yoon D, Agarwal AM, Mendell J, Prchal JT. Regulated expression of microRNAs in normal and polycythemia vera erythropoiesis. *Exp Hematol* 2007;**35**:1657–67.

90. Waddington CH. *The Strategy of the Genes. A Discussion of Some Aspects of Theoretical Biology*. London: Allen & Unwin; 1957.

91. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;**126**:663–76.

92. Takahashi K, Okita K, Nakagawa M, Yamanaka S. Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc* 2007;**2**:3081–9.

93. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007;**131**:861–72.

94. Meissner A, Wernig M, Jaenisch R. Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat Biotechnol* 2007;**25**:1177–81.

95. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 2007;**448**:313–17.

96. Hanna J, Wernig M, Markoulaki S, Sun. CW, Meissner A, Cassady JP, et al. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 2007;**318**:1920–3.

97. Aoi T, Yae K, Nakagawa M, Ichisaka T, Okita K, Takahashi K, et al. Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science* 2008;**321**:699–702.

98. Lowry WE, Richter L, Yachechko R, Pyle AD, Tchieu J, Sridharan R, et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci USA* 2008;**105**:2883–8.

99. Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol* 2008;**26**:101–6.

100. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008;**451**:141–6.

101. Wernig M, Meissner A, Cassady JP, Jaenisch R. c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* 2008;**2**:10–12.

102. Imamura M, Miura K, Iwabuchi K, Ichisaka T, Nakagawa M, Lee J, et al. Transcriptional repression and DNA hypermethylation of a small set of ES cell marker genes in male germline stem cells. *BMC Dev Biol* 2006;**6**:34.

103. Mikkelsen TS, Hanna J, Zhang X, Ku M, Wernig M, Schorderet P, et al. Dissecting direct reprogramming through integrative genomic analysis. *Nature* 2008;**454**:49–55.

104. Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K, et al. Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* 2007;**1**:55–70.

105. Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotech* 2008;**26**:1269–75.

106. Knoepfler PS, Zhang XY, Cheng PF, Gafken PR, McMahon SB, Eisenman RN. Myc influences global chromatin structure. *EMBO J* 2006;**25**:2723–34.

107. Kazantsev AG, Thompson LM. Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. *Nat Rev Drug Discov* 2008;7:854–68.

108. Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. *Nat Rev Drug Discov* 2006;**5**:37–50.

109. Viswanathan SR, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. *Science* 2008;**320**:97–100.

110. Rybak A, Fuchs H, Smirnova L, Brandt C, Pohl EE, Nitsch R, et al. A feedback loop comprising lin-28 and let-7 controls pre-let-7 maturation during neural stem-cell commitment. *Nat Cell Biol* 2008;**10**:987–93.