Review Article

Integration of Signaling Pathways with the Epigenetic Machinery in the Maintenance of Stem Cells

Luca Fagnocchi, Stefania Mazzoleni, and Alessio Zippo

Fondazione Istituto Nazionale di Genetica Molecolare “Romeo ed Enrica Invernizzi”, 20122 Milano, Italy

Correspondence should be addressed to Alessio Zippo; zippo@ingm.org

Received 28 May 2015; Revised 18 August 2015; Accepted 26 August 2015

Academic Editor: Aster H. Juan

Copyright © 2016 Luca Fagnocchi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Stem cells balance their self-renewal and differentiation potential by integrating environmental signals with the transcriptional regulatory network (TRN) [1–4]. Adult stem cells are generally long-lived quiescent cells, which, upon prodifferentiation stimuli, would give rise to progenitors that will further differentiate into postmitotic mature cells. Controlling the equilibrium between stem cell self-renewal and cell fate specification is indispensable for maintaining tissue homeostasis and the deregulation of these processes would lead to loss of cell identity and tumor initiation [5–7]. In the early embryo, the inner cell mass (ICM) cells are pluripotent and progressively restrict their developmental potential in response to local cues, which direct the formation of the three germinal layers. Defining the molecular mechanisms that govern the establishment of a defined epigenetic program in response to transient signals is fundamental to understand the basis of stem cell specification and reprogramming. The feasibility of isolating and propagating in culture both embryonic and adult stem cells, which can self-renew or differentiate in response to specific signals, allows delineating how extrinsic signals are integrated with the TRN [5, 8–10]. Signaling pathways crosstalk fine-tunes the correct pattern and timing of gene expression by modulating downstream effectors such as transcription factors (TFs), cofactors, and histones modifiers. These modulations are achieved through different mechanisms including differential DNA binding affinities, protein shuttling, posttranslational modifications, and protein-protein interactions. Importantly, the combinatorial DNA binding action of cell type-specific TFs and signal effectors on cis-regulatory elements is strongly influenced by the chromatin landscape of a given cell, thus resulting in the establishment of multiple transcriptional programs. In this regard, the dynamic interplay between signaling pathways, TFs, and epigenetic machinery plays a major role in integrating multiple inputs and switching a transient signaling event into a long-lasting phenotypic change.

In this review, we will discuss regulatory mechanisms through which signaling cascade can directly regulate histone modifications, nucleosome occupancy, and chromatin
modifying enzymes. We will highlight how these chromatin modifications triggered by extrinsic signaling may affect the TFs binding, the epigenetic state, and the consequent gene expression program of stem cells. Finally, we would underline the critical role of these regulatory circuits to control the cell identity and how their misregulation may initiate pathological events such as tumorigenesis.

2. Mechanisms of Signaling to Chromatin

2.1. Signaling Mechanisms Regulating Histone Modifications. The coordinated activation of signaling pathways impacts the epigenetic landscape by targeting TFs, chromatin regulators, or nucleosome occupancy or by directly modifying nucleosomes (Table 1).

Histones are subject to a large set of posttranslational modifications (PTMs) including phosphorylation, acetylation, methylation, ubiquitination, sumoylation, and citrullination, which influence the chromatin structure [11, 12]. The possible combinations of histone modifications differently affect the chromatin accessibility to TFs and determine molecular platforms for recruiting regulatory complexes, which would further modify the chromatin state. Importantly, histone modifications are reversible as opposing modifying enzymes, writers and eraser, introduce or remove the same modifications in response to specific signals [13–16]. Among them, kinases are activated mainly by upstream signaling cascade and they transiently phosphorylate both histone and nonhistone nuclear proteins [17, 18]. The temporal pattern of a certain pathway combined with the cell type-specific chromatin state strongly affects the resulting transcriptional outcome [19]. A large body of data shows that histone phosphorylation influences the deposition of other histone modifications facilitating the recruitment of Histone Acetyltransferases (HATs) while opposing the maintenance of repressive marks. Phosphorylation of histone H3 on serine 10 (H3S10ph) is accomplished in response to different signaling cascades, which activate the downstream kinases such as Rsk2, MSK1/2, IKKa, Aurora B, and PIM1 [14, 20–23]. Although the stimulus-induced H3S10ph is transient, it could cooperate with histone acetylation in blocking the binding of the chromodomain containing protein HP1α, thus promoting chromatin remodeling and transcription activation [23]. At enhancers, H3S10 phosphorylation drives the recruitment of MOF, which, by acetylating histone H4, establishes a nucleosome binding platform for the BRD4/P-TEFb complex, thereby stimulating transcription elongation [24]. Other histones phosphorylation is involved in controlling transcriptional switch by mediating histone crosstalk. For example, during androgen receptor- (AR-) dependent gene activation, PKCβ-mediated H3T6 phosphorylation switches the LSD1 demethylation activity from H3K4 towards H3K9 methyl group [25]. Similarly, the epidermal growth factor- (EGF-) activated tumor-specific pyruvate kinase M2 (PKM2) phosphorlates histone H3 at T11, which triggers dissociation of HDAC3, thus favoring H3K9ac and transcription activation [26].

Taken together, these results illustrate how a kinase-mediated short-lived signal activates a cascade of events, which determines a long-standing output by inducing chromatin modifications and impacting gene expression.

2.2. Signaling Mechanisms Regulating Nucleosome Occupancy. Nucleosome organization and higher order chromatin structures package genomic DNA, limiting its accessibility to most of the nuclear factors. Chromatin remodelers are multisubunit complexes that utilize ATP hydrolysis to mobilize nucleosomes and their positioning on the eukaryotic DNA, thereby being essential for modulating chromatin accessibility to transcription factors and RNA Polymerases [27, 28]. In addition, histone chaperones and DNA helicases facilitate histone exchange and the insertion of histone variants into nucleosomes surrounding cis-regulatory elements such as promoters and enhancers [29–32]. Many sophisticated mechanisms involve the crosstalk between signaling pathways and chromatin remodelers in order to alter nucleosome occupancy, as a requisite for gene regulation. The steroid hormone receptors interact and recruit SWI/SNF complexes to render the chromatin more accessible. In breast cancer cell, progesterone-activated ERK1/2 phosphorylates both the progesterone-receptor (PR) and the downstream kinase MSK1, forming an active ternary complex, which mediates the phosphorylation of histone H3 at serine 10. This initial step triggers the recruitment of histone modifiers and chromatin remodeling complexes, which ultimately leads to local displacement of histones H1 and H2A/H2B. In this setting, chromatin remodeling is responsible for transcriptional activation of progesterone responsive genes [33–35]. Another study linked nucleosome occupancy at enhancers to androgen receptor (AR) signaling [36]. Apart from nuclear receptors, other signaling pathways have been recently involved in modulating nucleosome occupancy. Specifically, it has been shown that the downstream effectors of the Hippo pathway YAP/TAZ promote transcriptional repression of numerous target genes by stimulating chromatin remodeling. YAP/TAZ interact with the TEAD transcription factor and recruit the NuRD complex on target genes, causing histones deacetylation and increased H3 histone occupancy, thus leading to chromatin compaction [37].

These data illustrate how chromatin remodelers are influenced by environmental signals, which in turn modulate nucleosome occupancy, thereby affecting transcription regulation.

2.3. Signaling Mechanisms Regulating Chromatin Modifiers. Signaling pathways can also impact the chromatin state by targeting chromatin modifying proteins. The activation of the Jak2/STAT5 pathway leads to Jak-dependent phosphorylation of STAT5, which causes its dimerization, nuclear translocation, and binding to cis-regulatory elements. In addition, Jak2 functions as histone tyrosine kinase by phosphorylating H3Y41 and perturbing HP1α binding [38].

Another example of linking signaling pathways with chromatin modifications is represented by the Polycomb and Trithorax group of proteins which act antagonistically in maintaining a specific gene expression state [39, 40]. The H3K27 methyltransferase enzyme EZH2 is the catalytic...
| Mechanism of signaling to chromatin | Signaling pathway | Chromatin target | Functional outcome | Reference |
|-----------------------------------|------------------|-----------------|--------------------|-----------|
| Histone posttranslational modifications | Serum stimulated PIM1 kinase cascade | H3S10 phosphorylation | Recruitment of MOF, which acetylates H4, thus in turn recruiting the BRD4/P-TEFb complex and stimulating transcription elongation | [14] |
| | Epidermal growth factor (EGF) induced Rsk2 kinase signaling | H3S10 phosphorylation | Recruitment of HAT complexes and rapid acetylation of phosphorylated H3S10 | [20] |
| | Mitogen- and stress-induced MSK1/2 cascade | H3S10 and S28 phosphorylation | Reduced efficiency in inducing mitogen- and stress-induced IE genes | [20] |
| | Cytokine stimulated IKKa kinase cascade | H3S10 phosphorylation | Regulation of NF-κB-dependent gene expression after cytokine exposure | [22] |
| | Mitotic Aurora B kinase signaling | H3S10 phosphorylation | Displacement of HP1 from mitotic heterochromatin and gene activation | [23] |
| | Androgen dependent PKCβ kinase signaling | H3T6 phosphorylation | Androgen-stimulated gene expression activation, through modulation of LSD1 demethylating activity | [25] |
| | Epidermal growth factor (EGF) activated PKM2 kinase cascade | H3T11 phosphorylation | Dissociation of HDAC3 from CCND1 and MYC promoters, introduction of H3K9ac, and induction of transcription activation | [26] |
| | Jak2/STAT5 signaling pathway | H3Y41 phosphorylation | Jak2 acts as histone tyrosine kinase, which phosphorylates H3Y41 and excludes HP1a from chromatin | [38] |
| Modulation of nucleosome occupancy | Progesterone-activated ERK1/2 signaling | Histones H1 and H2A/H2B | ERK1/2 mediated phosphorylation of the progesterone receptors, MSK1 and H3S10, which recruit chromatin remodeling complexes leading to the displacement of H1 and H2A/H2B and transcriptional activation of progesterone responsive genes | [33–35] |
| | Androgen signaling pathway | Nucleosomes | Induction of a nucleosome-depleted state at androgen receptor enhancers, leading to recruitment of histone modifiers, chromatin remodelers, and ultimately gene activation | [66] |
| | Hippo signaling pathway | Histones H3 | The YAP/TAZ/TEAD ternary complex recruits NuRD complex on target genes, leading to histones deacetylation, increased H3 histone occupancy and reduction of chromatin accessibility | [37] |
| Regulation of chromatin modifiers | Stress-activated p38α kinase cascade | EZH2 Thr372 phosphorylation | PRC2-mediated repression of Pax7 during regeneration | [41] |
| | PI3K-AKT signaling pathway | EZH2 Ser21 phosphorylation | Suppression of EZH2 methyltransferase activity by reducing its binding to histone H3 and derepression of silenced genes | [42] |
| | p38 MAPK signaling pathway | MLL complexes | The signaling cascade leads to phosphorylation of Mef2c, which interacts with MLL complex, targeting it to specific genes that are activated during myogenesis | [43] |

Subunit of the polycomb repressive complex 2 (PRC2) and is targeted by different signals, which can promote or inhibit its enzymatic activity, respectively [41, 42]. The stress-activated p38α kinase phosphorylates EZH2 on Thr372 in muscle satellite cells and promotes PRC2-mediated repression of Pax7 during myogenesis. Instead, the prosurvival PI3K-AKT signaling pathway targets EZH2 by inducing Ser21 phosphorylation, which causes the reduction of PRC2 affinity for histone H3. At the same time, AKT-mediated phosphorylation of P300 increases its H3K27-specific acetyltransferase activity, thus participating in switching from a methyl (repressive) towards an acetylated (active) K27 state.
On the other hand, Myeloid/Lymphoid or Mixed-Lineage Leukemia (MLL) group of proteins mediates the trimethylation of histone 3 at lysine 4 (H3K4me3) and are core components of the Trithorax complexes. Multiple MLLs are targeted in response to signaling leading to their PTMs. For example, during the commitment of myoblasts into multinucleated myotubes, p38 MAPK signaling pathway leads to phosphorylation of Mef2d and its interaction with MLL2 complex. This signaling cascade promotes MLL2 targeting to muscle-specific genes leading to their H3K4 trimethylation complex. This signaling cascade promotes MLL2 targeting to muscle-specific genes leading to their H3K4 trimethylation and transcriptional activation [43].

Overall, the reported examples clearly show that signaling cascades not only influence the activity of transcription factors but also perturb the chromatin state by driving dynamic chromatin changes that impact on the transcriptional program.

3. Outcomes of Integrated Signals on Stem Cells Transcriptional and Epigenetic State

Beside the examples described so far, developmental signaling pathways are also interconnected with the TRN and influence the chromatin state of stem cells (Figure 1). The developmental signaling, which includes the Wnt/β-catenin, Notch, Nodal/Activin, Hippo pathways, and the circadian clock, is involved both in the maintenance of stem cell homeostasis and in inducing cell lineage commitment. In general, their activation triggers the stabilization and the nuclear accumulation of their downstream effectors, which finally influence the expression of their target genes.

The downstream effectors, which are activated in a controlled spatiotemporal manner by the external stimuli, provide the competence for a stem cell to adopt a particular cell fate by cooperating with the cell type-specific TFs. This concept is particularly relevant in pluripotent embryonic stem cells (ESCs), in which the same signaling pathways play a key role in the maintenance of self-renewal capacity but are also involved in lineage differentiation. This divergent stem cell responsiveness depends on the fact that signaling pathways target both TRNs and chromatin landscapes. Besides that, the integration of multiple extrinsic signals determines different transcriptional program, thus influencing the cellular response.

3.1. Signaling to the Transcriptional Regulatory Network of Embryonic Stem Cells. Both mouse and human ESCs (mESCs and hESCs) are isolated from the transient pluripotent cells of the inner cell mass (ICM) [44, 45]. The two major features that define ESCs consist in their ability to self-renew as well as to differentiate into all the cell lineages in response to developmental cues. This balance is regulated by a specific transcription program, which is centered on the cooperative action of the pluripotency transcription factors Oct4, Sox2, and Nanog (OSN) [46]. OSN targets have been mapped and showed an extensive co-binding in both mESCs and hESCs, suggesting the existence of a common core transcriptional regulatory network (TRN) [47, 48]. Oct4 is a member of the POU family of homeodomain proteins and it is essential for the establishment and maintenance of pluripotency both in vivo and in vitro. Perturbation of Oct4 transcript level abrogates formation of the ICM [49] and promotes ESCs differentiation [50]. Oct4 heterodimerizes with the high-mobility group box (HMG) family member Sox2 and they co-bind distal regulatory elements, thus activating the expression of many pluripotency factors and repressing lineage-specific genes. The synergic action of Oct4/Sox2 in the regulation of key pluripotency factors is underlined by the similar phenotype observed both during blastocyst formation and in cultured ESCs upon the knock-out of the respective genes [51, 52]. Although Nanog is not essential for deriving and maintaining ESCs, it is required for the formation of the ICM. Functionally, Nanog cooccupies most sites with Oct4/Sox2, thus playing a key role in controlling pluripotency in ESCs [53–55]. These core transcription factors control the ESCs transcriptional program by establishing an interconnected regulatory loop in which they influence the gene expression level of each other. This self-sustained transcription regulatory network generates a bimodal transcriptional state of ESCs, which is characterized by the coexistence of transient and exchangeable cellular states. Appropriate levels of the core transcription factors ensure a residence state in which ESCs self-renew. On the contrary, transient perturbation of the positive feedback transcriptional program produces a window of opportunity to exit pluripotency and to initiate cell lineage commitment [55–57]. The ability of OSN to maintain mESCs state is influenced by additional transcription factors such as Klf4, Klf2, Dax1, Nac1, Zfp281, Essrb, Sall4, Tbx3, and Pdm14, which co-bind enhancers occupied by OSN [3, 58–60]. Importantly, the OSN-centered regulatory network includes also Stat3, Smad1, and Tcf3, which are the downstream effectors of the LIF, BMP4, and Wnt signaling pathways [3, 61, 62]. These observations underline how the extracellular signals converge on the core TRN, thus participating in the modulation of the stem cell transcriptional program (Figures 1(a) and 1(b)). While LIF leads to phosphorylation of Stat3, which is required to promote self-renewal, BMP4 suppresses differentiation through Smad1-mediated activation of Id genes. Wnt signaling counteracts the transcriptional repressive activity of Tcf3 on pluripotency genes by stabilizing β-catenin [63, 64] (Figure 1(a)). However, both hESCs and mouse postimplantation epiblasts derived stem cells (EpiSCs), collectively referred to as “primed” pluripotent stem cells, depend on different signaling pathways for self-renewal, such as FGF/ERK and Activin A/Smad [65, 66] (Figure 1(b)). In particular, both the Wnt/β-catenin pathway and the BMP/Smad signaling cascades, which are required to promote mESCs pluripotency, once activated in primed stem cells trigger mesendoderm lineage commitment. In hESCs, nuclear β-catenin cooperates with SMAD2/3, leading to the activation of differentiation genes, thus inducing exit from pluripotency [67]. On the other hand, it has been shown that BMP4/TGF-β stimulation induces hESCs and EpiSC to differentiate towards mesoderm [68].

3.2. Signaling to Chromatin in Embryonic Stem Cells. Signaling-mediated gene regulation in ESCs could be directly
achieved through the modulation of chromatin players and the epigenetic machinery (Figure 1(c)).

In mESCs, a LIF-independent role for Jak signaling has been demonstrated and consists in the phosphorylation of histone H3 on tyrosine 41 (H3Y41). This event leads to a reduction in the binding of heterochromatin protein 1 (HP1) on pluripotency genes [69]. In hematopoietic stem cells, mutations leading to the activation of Jak2 correlate with myeloproliferative neoplastic and leukemic transformation. One such mutation is represented by the Jak2V617F allele, which turns on the Jak/STAT pathway without the requirement of activating cytokines [70, 71]. Interestingly,
the expression of Jak2V617F in mESCs leads to their cytokine independent self-renewal and is associated with the direct Jak2 signaling to the chromatin. Chemical inhibition of the Jak/STAT pathway in Jak2V617F mESCs leads to the decrease of H3Y41ph levels coupled with increased association of HP1α to Nanog promoter, thereby inducing its transcriptional repression. These findings underline the critical role of the direct Jak2 signaling to the chromatin in sustaining self-renewal of both embryonic and hematopoietic stem cells and how its deregulation may cause tumorigenesis [69]. In the same oncogenic setting, mutated Jak2 may also phosphorylate and inhibit PRMT5 preventing histone arginine methylation and favoring uncontrolled haematopoietic progenitor cell expansion [72]. Finally, both Jak2V617F and Jak2K539L, other oncogenic forms of Jak2, cooperate with the histone demethylase JMJD2C in lymphomas, by promoting MYC overexpression [73]. Contrary to the role of Jak2 on chromatin, MAP kinases signaling favors mESCs differentiation through the JNK-mediated H3 Ser10 (H3S10) phosphorylation of its target genes [74].

Other mechanisms of signaling to chromatin involve the modulation of the targeting of chromatin complexes. ERK pathway regulates PRC2 deposition at developmental genes, by phosphorylating the RNA polymerase II at serine 5 and establishing poised domains [75]. The chromatin remodeling complex esBAF is, instead, interconnected with the LIF/Stat3 signaling pathway. Brkl, the ATPase subunit of esBAF, favors the correct targeting of Stat3 onto chromatin by stimulating chromatin remodeling at Stat3 target genes, thus supporting mESCs pluripotency [61]. Similar mechanisms are shared between ESCs and cancer cells and are relevant for tumorigenesis. In ESCs, the pluripotency genes Myc and LIN28 counteract the action of Let-7, which inhibits self-renewal genes [76, 77]. Among others, HMGA2 represents a DNA binding and chromatin modifying protein which regulates both differentiation and stem cell self-renewal [78, 79]. Misregulation of the components of this regulatory circuit has been associated with a wide range of malignancies [80, 81]. Interestingly, in breast cancer cells, inhibition of the MAPK signaling by the Raf kinase inhibitory protein (RKIP) is transduced onto the chromatin where the HMGA2 activity is inhibited, leading to inactivation of proinvasive and prometastatic genes [82]. In hESCs, the Activin A/Smad pathway has been demonstrated to be involved in the correct deposition of H3K4me3 on key developmental genes, through its effectors SMAD2/3, which cooperate with NANOG to recruit DPY30, a subunit of the COMPASS methyltransferase complexes, contributing to the capacity of stem cells to differentiate into specific lineages [83].

3.3. Gene Expression Heterogeneity of ESCs and Fluctuating Signaling. Single-cell studies on mESCs showed substantial gene expression heterogeneity with subpopulations of ESCs, which express variegated levels of pluripotency-associated factors [35, 56, 84–87]. The discovery of fluctuating expression levels of pluripotency regulators, which supports the existence of interconvertible ESCs states with different potency to self-renew or differentiate, highlights the key role of sustaining a dynamic transcriptional program in pluripotent cells [87]. Fluctuations in gene expression may depend on multiple factors, which include the structure of the cell TRN, sequential and combinatorial epigenetic regulations, and the integration of signaling pathways.

The TRNs are characterized by recurring regulatory circuits, named network motifs, which define a particular pattern of interconnections, leading to a certain transcriptional outcome [88]. Among them, negative feedback loops and type 1 incoherent feedforward loops may generate oscillatory responses of TFs. Specifically, the ESCs regulatory circuit is characterized by dynamic TFs that regulate each other and autoregulate their own expression through both feedforward and negative feedback loops, thus determining fluctuating states of transcript levels within the ESC population [1, 48, 59, 60, 89–92]. At the posttranscriptional level, microRNAs (miRNAs) play a central role in modulating TRN as the core pluripotency factors OSN and Tcf3 directly bind their loci, thus influencing their expression [93]. Mechanistically, the ES cell-specific cycle-regulating (ESCC) miRNAs indirectly activate several self-renewal genes including c-Myc and Lin28 which, by inducing degradation of pre-Let-7 transcripts, inhibit Let-7 opposing effects on ESCs self-renewal [76, 77]. These results suggest that let-7 and ESCC miRNAs act in self-reinforcing loops to sustain the ESCs transcriptional network. The finding that many miRNAs target the pluripotency TFs in ESCs suggests that they may be implicated in controlling their fluctuating transcript levels.

Recently, it has been shown that impairment of miRNAs production in ESCs (Dgcr8−/− and Dicer−/− ESCs) resulted in a more homogenous expression of pluripotency factors [87]. In terms of transcription heterogeneity, the Dgcr8−/− ESCs manifest features similar to the so-called 2i ESCs, which mirror the “naïve” or “ground state” of preimplantation epiblast cells [2, 94]. The 2i ESCs are grown in a chemically defined medium which comprises the Mek inhibitor PD03 (PD0325901) and the GSK3 inhibitor ChIRON (CHIR99021), which shield ESCs from differentiation-autocrine signaling and reinforce for pro-self-renewing pathways [2] (Figure 1(a)). The Fgf4/Erk cascade drives the transition from naïve pluripotency to a primed state, which is responsive to lineage-specific differentiation signals [95]. GSK3 inhibition reinforces the Wnt pathway by stabilizing β-catenin [64, 96–98]. In 2i ESCs, the fluctuating expression of the pluripotency-associated transcription factors is strongly reduced, thus highlighting the crucial role of signaling pathways in modulating transcriptional pulsing in ESCs.

These observations could be explained by considering the intrinsic feature of signaling pathways, which is the dynamics. This represents an additional mode of transmitting information, meaning that signaling pathways encode information in the frequency, amplitude, and duration of the signals into the cells [99]. Importantly, cells are able to decode the signaling dynamics by executing different biological responses. For example, studying the ERK pathway revealed that different upstream signals trigger divergent dynamic patterns of the same signaling cascade leading to two different cellular fates [100]. In this case, the EGF treatment of PC-12 neural precursors drives a transient ERK
activation, which induces cell proliferation, whereas the NGF stimulus triggers a sustained ERK response, culminating in differentiation. The differences in ERK dynamics, in response to those alternative growth factors, depend on the network structure, which encodes the stimulus into a dynamic change of ERK activation. In the EGF pathway, the stimulus activates a SOS-dependent negative feedback loop. Instead, the NGF signal induces a PKC-centered positive feedback loop, thus sustaining ERK activation [101, 102]. The molecular mechanisms through which cells decode the temporal pattern of a certain signaling are poorly understood. Regarding the ERK dynamics, it has been proposed that the interpretation of the temporal signals is depending on network motifs that sense the spatiotemporal changes of the upstream signaling [103–105]. In this case, transient ERK activation leads to the expression of the immediate early gene c-Fos that is rapidly degraded. On the contrary, a persistent nuclear ERK signal drives the accumulation of the effector, which is directly phosphorylated by ERK itself, thus increasing its protein stability.

In pluripotent stem cells (PSCs), the ERK pathway triggers opposite cellular response: in na¨ıve ESCs, it induces cell lineage commitment while it sustains self-renewal of primed EpiSCs (Figures 1(a) and 1(b)). This striking difference could depend on the dissimilar cellular and epigenetic context of these PSCs but it may also be caused by diverse signaling dynamics, which are decoded differently, thus leading to divergent cellular fates (Figure 2). Few studies addressed this specific point in ESCs but the obtained results clearly showed a link between ERK signaling pathway and fluctuating transcriptional response [2, 94, 106, 107]. The attenuation of the autocrine Fgf4/MAPK signaling induced by either the chemical inhibition of Mek or the genetic targeting of the heparin sulfate proteoglycans reduces the transcriptional fluctuations of pluripotent transcription factors. Although the dynamics of autocrine FGF signaling has not been studied in mESCs, computational modeling, based on the well-studied EGF signaling in other systems, postulates that the oscillatory pattern of Nanog could depend on the dynamics of the regulatory system, including individual cell-specific changes in parameters of FGF autocrine feedback loop and crosstalk with other signaling pathways. The interconnection between MAPK, PI3K/AKT, BMP4/Nodal, and Wnt signaling plays a major role in the maintenance of hESCs [67]. Of note, the dynamics of these signaling pathways have been described to play a major role in controlling ESC pluripotency and reprogramming [108–112]. Among them, the Wnt/𝛽-catenin pathway is particularly interesting as its periodic activation favors cell fusion-mediated reprogramming while its sustained stimulation inhibits it [112]. Mechanistically, the activation of the Wnt signaling in the early stage of reprogramming causes a TCFI-dependent inhibitory effect, while its stimulation in the late phase reinforces reprogramming towards PSCs [110, 113]. It would be interesting to evaluate whether a similar fluctuating Wnt signaling pattern may support the maintenance of the naïve state in ESCs. However, recent data showed that β-catenin fluctuates in both “primed” (serum + LIF maintained) and naïve (2i + LIF maintained) mESCs [111]. These results are of particular interest considering that in the 2i condition the inhibition of GSK3-β should stabilize the endogenous β-catenin, thus suggesting that other regulatory circuits may modulate the dynamics of Wnt/β-catenin pathway.

More broadly, the observed transcriptional dynamics of pluripotency-associated TFs may reflect the integration of input at the chromatin level including histone modification, chromatin accessibility, the topology of the transcriptional regulatory networks, and activity of autocrine signaling pathways. Despite their importance, the effects of these fluctuating signaling pathways at the transcriptional and chromatin level have not been investigated so far. For example, there are no data regarding the dynamic response of the downstream effectors of the fluctuating signaling pathways in stem cells, nor on the impact on histone modifications at the target genes. Although understanding how cells decode the different dynamical patterns at the molecular level is currently a challenging goal, it is mandatory to better define these regulatory mechanisms in order to clarify their contribution to the maintenance of stem cell identity and pluripotency.

3.4. Transcriptional Dynamics in Neural Progenitor Cell Fate Choice. The importance of the integration between signaling dynamics, TRN, and the epigenetic state is well exemplified during cell lineage choice of pluripotent Neural Progenitor Cells (NPCs) in developing nervous system. In the developing telencephalon, the neuroepithelial cells, which represent the earliest NPCs, proceed towards the formation of Radial Glial (RG) cells by the oscillating Notch signaling [114–116]. Asymmetric cell division of polarized RG cells gives rise to immature neurons which would further differentiate into mature neurons and to intermediate progenitors, which go towards cell division in the subventricular zone (SVZ) before fully differentiating. During neurogenesis, it is essential to maintain a certain balance between self-renewing NPCs, proliferating intermediate progenitors, and their commitment towards postmitotic differentiated cells [114]. This goal is achieved, at least in part, by integrating the fluctuating Notch signaling and the transcriptional regulatory circuit of NPCs. In particular, the Notch pathway induces the expression of the bHLH transcription factors Hesl and Hes5, which are required for the specification of RG cells [117, 118]. Of interest, in neural progenitors, these factors are expressed in an oscillatory manner in response to the fluctuating expression of the Notch ligand Dll1, as well as a consequence of their negative feedback loop. The Hes transcription factors maintain the precursors’ multipotency by inhibiting the proneural bHLH factors Ascl1 and Ngn2 [118]. The two bHLH transcription factors Olig1/2 are required to specify the formation of oligodendrocyte progenitor cells and their subsequent differentiation and maturation. Astrocytes fate determination is the result of the interplay between transcription factors, epigenetic modifiers, and environmental signals. Specifically, during neurogenesis, NSCs become responsive to Jak/STAT and BMP signaling pathways, which support astrocyte differentiation, as a consequence of transcription factors-dependent DNA and histone demethylation of the astrocyte-specific genes [119].
Figure 2: Emergence of gene expression heterogeneity in ESCs and cell fate determination. Gene expression heterogeneity of ESCs is determined by complex multistep mechanisms. (a) Multiple spatiotemporal restricted signals are differentially sensed and integrated by ESCs, leading to signaling pathways activation, which ultimately converges both onto the TRN and directly onto the chromatin. (b) Specific regulatory networks, which involve both TFs and epigenetic regulators, are established in the cell according to their transcriptional and epigenetic landscape and transduce the signals. Arrows and lines indicate positive and negative regulation between factors (black circles), respectively. Negative feedback loops (left) or incoherent feedforward loops (right) may generate oscillatory responses to signals. (c) The result of this integration is the fluctuation of genes expression profiles among cells, which permits ESCs to fluctuate in a continuum of interconvertible pluripotent states and may generate the suitable condition to exit pluripotency and differentiate. (d) The final biological outcome of this process is the establishment of a heterogeneous population of ESCs captured at different pluripotent states (green and purple cells) or the eventual differentiation toward committed cell (blue cell).
The lineage-commitment factors Ascl1, Hes1, and Olig2 play opposite function in sustaining proliferation and cell differentiation of NPCs [120–123]. This contradictory function can be explained by considering their dynamic pattern rather than their relative transcriptional level. Live cell imaging studies have shown that the Notch-dependent fluctuating pattern of Hes1 causes oscillation of Ascl1 and Ngn2 in neural precursors [124–126]. Of importance, by adapting an optogenetic approach to mimic the spatiotemporal pattern of Ascl1 expression in NPCs, it has been demonstrated that periodic oscillations of this TF induce cell proliferation, while its prolonged transcriptional activation triggers lineage commitment towards the formation of neurons [126]. The molecular mechanism through which NPCs differentially interpret the dynamics of Ascl1 gene expression is currently undefined. In addition, it has not been determined which are the different targets that are responsive to this encoded information. Moreover, it has not been investigated so far whether this expression dynamics may be integrated into the chromatin, giving rise to different pattern of histone modifications in the two opposite settings (fluctuating versus sustained transcription).

4. Conclusions and Future Perspectives

Over the recent past years, the massive utilization of systems biology techniques and functional genomics increased dramatically our knowledge on the regulatory networks, which control both the maintenance of cell identity and the lineage commitment. Nonetheless, a better understanding of how cells integrate multiple environmental signals and transduce them onto chromatin, in order to modulate gene expression, is still needed.

In this review, we provide multiple evidences, demonstrating how different pluripotent stem cells rely on specific extrinsic cues, which converge on transcriptional and epigenetic networks, thereby determining their cell fate (Figures 1 and 2). Pluripotency is not an invariant state, but rather represents a continuum of states between which cells can integrate multiple environmental signals and transduce them onto chromatin, in order to modulate gene expression, is still needed.

The authors declare that there is no conflict of interests regarding the publication of this paper.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] H.-H. Ng and M. A. Surani, “The transcriptional and signalling networks of pluripotency,” Nature Cell Biology, vol. 13, no. 5, pp. 490–496, 2011.
[2] Q.-L. Ying, J. Wray, J. Nichols et al., “The ground state of embryonic stem cell self-renewal,” Nature, vol. 453, no. 7194, pp. 519–523, 2008.
[3] X. Chen, H. Xu, P. Yuan et al., “Integration of external signaling pathways with the core transcriptional network in embryonic stem cells,” Cell, vol. 133, no. 6, pp. 1106–1117, 2008.
[4] H. Clevers, K. M. Loh, and R. Nusse, “An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control,” Science, vol. 346, no. 6205, Article ID 1248012, 2014.
[5] M. Petersson, K. Reuter, H. Brylka, A. Kraus, P. Schettina, and C. Niemann, “Interfering with stem cell-specific gatekeeper functions controls tumour initiation and malignant progression of skin tumours,” Nature Communications, vol. 6, p. 5874, 2015.
[6] L. Vermeulen, E. Morrissey, M. Van Der Heijden et al., “Defining stem cell dynamics in models of intestinal tumor initiation,” Science, vol. 342, no. 6161, pp. 995–998, 2013.
[7] S. Schwiattala, A. A. Fingerle, P. Cammareri et al., “Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties,” Cell, vol. 152, no. 1-2, pp. 25–38, 2013.
[8] T. Sato, R. G. Vries, H. J. Snippert et al., “Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche,” Nature, vol. 459, no. 7244, pp. 262–265, 2009.
[9] S. P. Boj, C.-I. Hwang, L. A. Baker et al., “Organoid models of human and mouse ductal pancreatic cancer,” Cell, vol. 160, no. 1-2, pp. 324–338, 2015.

[10] J.-C. Yeo and H.-H. Ng, “The transcriptional regulation of pluripotency,” Cell Research, vol. 23, no. 1, pp. 20–32, 2013.

[11] T. Kouzarides, “Chromatin modifications and their function,” Cell, vol. 128, no. 4, pp. 693–705, 2007.

[12] P. Tessarz and T. Kouzarides, “Histone core modifications regulating nucleosome structure and dynamics,” Nature Reviews Molecular Cell Biology, vol. 15, no. 11, pp. 703–708, 2014.

[13] V. M. Weake and J. L. Workman, “Inducible gene expression: diverse regulatory mechanisms,” Nature Reviews Genetics, vol. 11, no. 6, pp. 426–437, 2010.

[14] A. Zippo, A. De Robertis, R. Serafini, and S. Oliviero, “PIM1-dependent phosphorylation of histone H3 at serine 10 is required for MYC-dependent transcriptional activation and oncogenic transformation,” Nature Cell Biology, vol. 9, no. 8, pp. 932–944, 2007.

[15] F. De Santa, M. G. Totaro, E. Prosperini, S. Notarbartolo, G. Testa, and G. Natoli, “The histone H3 lysine-27 demethylase JmjD3 links inflammation to inhibition of polyclomb-mediated gene silencing,” Cell, vol. 130, no. 6, pp. 1083–1094, 2007.

[16] W. A. Whyte, S. Bilodeau, D. A. Orlando et al., “Enhancer decomposition by LSD1 during embryonic stem cell differentiation,” Nature, vol. 482, no. 7384, pp. 221–225, 2012.

[17] S. Hu, Z. Xie, A. Onishi et al., “Profiling the human protein-DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling,” Cell, vol. 139, no. 3, pp. 610–622, 2009.

[18] S. H. Baek, “When signaling kinases meet histones and histone modifiers in the nucleus,” Molecular Cell, vol. 42, no. 3, pp. 274–284, 2011.

[19] J. E. Toettcher, O. D. Weiner, and W. A. Lim, “Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module,” Cell, vol. 155, no. 6, pp. 1422–1434, 2013.

[20] P. Cheung, K. G. Tanner, W. L. Cheung, P. Sassone-Corsi, J. M. Denu, and C. D. Allis, “Synergistic coupling of histone H3 phosphorylation and acetylation in response to epidermal growth factor stimulation,” Molecular Cell, vol. 5, no. 6, pp. 905–915, 2000.

[21] A. Soloaga, S. Thomson, G. R. Wiggins et al., “MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14,” The EMBO Journal, vol. 22, no. 11, pp. 2788–2797, 2003.

[22] V. Anest, J. L. Hanson, P. C. Cogswell, K. A. Steinbrecher, B. D. Strahl, and A. S. Baldwin, “A nucleosomial function for Ikb kinase-a in NF-kB-dependent gene expression,” Nature, vol. 423, no. 6940, pp. 659–663, 2003.

[23] T. Hirota, J. J. Lipp, B.-H. Toh, and J.-M. Peters, “Histone H3 serine 10 phosphorylation by Aurora B causes HP1 dissociation from heterochromatin,” Nature, vol. 438, no. 7071, pp. 1176–1180, 2005.

[24] A. Zippo, R. Serafini, M. Rocchigiani, S. Pennacchini, A. Krepeleva, and S. Oliviero, “Histone crosstalk between H3S10ph and H4K16ac generates a histone code that mediates transcription elongation,” Cell, vol. 138, no. 6, pp. 1122–1136, 2009.

[25] E. Metzger, A. Imhof, D. Patel et al., “Phosphorylation of histone H3T6 by PKCβ2 controls demethylation at histone H3K4,” Nature, vol. 464, no. 7289, pp. 792–796, 2010.

[26] W. Yang, Y. Xia, D. Hawke et al., “PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis,” Cell, vol. 150, no. 4, pp. 685–696, 2012.

[27] K. Bouazoune, T. B. Miranda, P. A. Jones, and R. E. Kingston, “Analysis of individual remodeled nucleosomes reveals decreased histone-DNA contacts created by hSW1/SNF,” Nucleic Acids Research, vol. 37, no. 16, Article ID gkp524, pp. 5279–5294, 2009.

[28] Y. Lorch, M. Zhang, and R. D. Kornberg, “Histone octamer transfer by a chromatin-remodeling complex,” Cell, vol. 96, no. 3, pp. 389–392, 1999.

[29] G. Mizuguchi, X. Shen, J. Landry, W.-H. Wu, S. Sen, and C. Wu, “ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex,” Science, vol. 303, no. 5656, pp. 343–348, 2004.

[30] M. Papamichos-Chronakis, S. Watanabe, O. J. Rando, and C. L. Peterson, “Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity,” Cell, vol. 144, no. 2, pp. 200–213, 2011.

[31] M. P. Schnett, L. Handoko, B. Akhtar-Zaidi et al., “CHD7 targets active gene enhancer elements to modulate ES cell-specific gene expression,” PLoS Genetics, vol. 6, no. 7, Article ID e1001023, 2010.

[32] M. L. Dechassa, A. Sabri, S. Pondugula et al., “SWI/SNF has intrinsic nucleosome disassembly activity that is dependent on adjacent nucleosomes,” Molecular Cell, vol. 38, no. 4, pp. 590–602, 2010.

[33] G. P. Vicent, A. S. Nacht, R. Zaurin et al., “Unliganded p53-gestressor receptor-mediated targeting of an RNA-containing repressive complex silences a subset of hormone-inducible genes,” Genes & Development, vol. 27, no. 10, pp. 1179–1197, 2013.

[34] G. P. Vicent, A. S. Nacht, J. Font-Mateu et al., “Four enzymes cooperate to displace histone H1 during the first minute of hormonal gene activation,” Genes & Development, vol. 25, no. 8, pp. 845–862, 2011.

[35] R. H. G. Wright, G. Castellano, J. Bonet et al., “CDK2-dependent activation of PARP-1 is required for hormonal gene regulation in breast cancer cells,” Genes & Development, vol. 26, no. 17, pp. 1972–1983, 2012.

[36] A. V. Claudia, J. Lai, B. P. Berman et al., “Dynamic nucleosome-depleted regions at androgen receptor enhancers in the absence of ligand in prostate cancer cells,” Molecular and Cellular Biology, vol. 31, no. 23, pp. 4648–4662, 2011.

[37] M. Kim, T. Kim, R. L. Johnson, and D. S. Lim, “Transcriptional co-repressor function of the hippo pathway transducers YAP and TAZ,” Cell Reports, vol. 11, pp. 270–282, 2015.

[38] M. A. Dawson, A. J. Bannister, B. Göttingens et al., “JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin,” Nature, vol. 461, no. 7265, pp. 819–822, 2009.

[39] E. R. Smith, G. L. Min, B. Winter et al., “Drosophila UTX is a histone H3 Lys27 demethylase that colocalizes with the elongating form of RNA polymerase II,” Molecular and Cellular Biology, vol. 28, no. 3, pp. 1041–1046, 2008.

[40] V. Narendra, P. P. Rocha, D. An et al., “CTCF establishes discrete functional chromatin domains at the Hox clusters during differentiation,” Science, vol. 347, no. 6225, pp. 1017–1021, 2015.

[41] D. Palacios, C. Mozzetta, S. Consalvi et al., “TNF/p38 signaling to PAX7 locus in satellite cells links inflammation to the epigenetic control of muscle regeneration,” Cell Stem Cell, vol. 7, no. 4, pp. 455–469, 2010.

[42] T.-L. Cha, B. P. Zhou, W. Xia et al., “Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3,” Science, vol. 310, no. 5746, pp. 306–310, 2005.
[43] S. Rampalli, L. Li, E. Mak et al., “p38 MAPK signaling regulates recruitment of Ash2L-containing methyltransferase complexes to specific genes during differentiation,” Nature Structural & Molecular Biology, vol. 14, no. 12, pp. 1150–1156, 2007.

[44] M. J. Evans and M. H. Kaufman, “Establishment in culture of pluripotent cells from mouse embryos,” Nature, vol. 292, no. 5819, pp. 154–156, 1981.

[45] J. A. Thomson, “Embryonic stem cell lines derived from human blastocysts,” Science, vol. 282, no. 5391, pp. 1145–1147, 1998.

[46] R. A. Young, “Control of the embryonic stem cell state,” Cell, vol. 144, no. 6, pp. 940–954, 2011.

[47] L. A. Boyer, I. L. Tong, M. F. Cole et al., “Core transcriptional network regulates pluripotency in mouse embryonic stem cells,” Nature, vol. 482, no. 7388, pp. 410–423, 2012.

[48] J. Nichols, B. Zevnik, K. Anastassiadis et al., “Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4,” Cell, vol. 95, no. 3, pp. 379–391, 1998.

[49] H. Niwa, J.-I. Miyazaki, and A. G. Smith, “Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells,” Nature Genetics, vol. 24, no. 4, pp. 372–376, 2000.

[50] A. A. Avilion, S. K. Nicolas, L. H. Pevny, L. Perez, N. Vivian, and R. Lovell-Badge, “Multipotent cell lineages in early mouse development depend on SOX2 function,” Development, vol. 17, no. 1, pp. 126–140, 2003.

[51] S. Masui, Y. Nakatake, Y. Toyooka et al., “Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells,” Nature Cell Biology, vol. 9, no. 6, pp. 625–635, 2007.

[52] K. Mitsui, Y. Tokuzawa, H. Itoh et al., “The homeoprotein nanog is required for maintenance of pluripotency in mouse epiblast and ES cells,” Cell, vol. 113, no. 5, pp. 631–642, 2003.

[53] J. Silva, J. Nichols, T. W. Theunissen et al., “Nanog is the gateway to the pluripotent ground state,” Cell, vol. 138, no. 4, pp. 722–737, 2009.

[54] I. Chambers, J. Silva, D. Colby et al., “Nanog safeguards pluripotency and mediates germline development,” Nature, vol. 450, no. 7173, pp. 1230–1234, 2007.

[55] Y. Toyooka, D. Shimosato, K. Murakami, K. Takahashi, and H. Niwa, “Identification and characterization of subpopulations in undifferentiated ES cell culture,” Development, vol. 135, no. 5, pp. 909–918, 2008.

[56] V. Karwacki-Neisius, J. Gøke, R. Osorno et al., “Reduced Oct4 expression directs a robust pluripotent state with distinct signaling activity and increased enhancer occupancy by Oct4 and Nanog,” Cell Stem Cell, vol. 12, no. 5, pp. 531–545, 2013.

[57] J. Kim, J. Chu, X. Shen, J. Wang, and S. H. Orkin, “An extended transcriptional network for pluripotency of embryonic stem cells,” Cell, vol. 132, no. 5, pp. 1049–1061, 2008.

[58] C. Y. Lim, W.-L. Tam, J. Zhang et al., “Sall4 regulates distinct transcription circuits in different blastocyst-derived stem cell lineages,” Cell Stem Cell, vol. 3, no. 5, pp. 543–554, 2008.

[59] J. Jiang, Y.-S. Chan, Y.-H. Loh et al., “A core Klf circuitry regulates self-renewal of embryonic stem cells,” Nature Cell Biology, vol. 10, no. 3, pp. 353–360, 2008.

[60] L. Ho, E. L. Miller, J. L. Ronan, W. Q. Ho, R. Jothi, and G. R. Crabtree, “esBAF facilitates pluripotency by conditioning the genome for LIF/STAT3 signalling and by regulating polycomb function,” Nature Cell Biology, vol. 13, no. 8, pp. 903–913, 2011.

[61] M. F. Cole, S. E. Johnstone, J. J. Newman, M. H. Kagey, and R. A. Young, “Tcf3 is an integral component of the core regulatory circuitry of embryonic stem cells,” Genes & Development, vol. 22, no. 6, pp. 746–755, 2008.

[62] H. Niwa, T. Burdon, I. Chambers, and A. Smith, “Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3,” Cell Stem Cell, vol. 12, no. 13, pp. 2048–2060, 1998.

[63] J. Wray, T. Kalkan, S. Gomez-Lopez et al., “Inhibition of glycogen synthase kinase-3 alleviates Tcf3 repression of the pluripotency network and increases embryonic stem cell resistance to differentiation,” Nature Cell Biology, vol. 13, pp. 838–845, 2011.

[64] J. A. Thomson, “Embryonic stem cell lines derived from human embryos,” Nature Genetics, vol. 144, no. 6, pp. 940–954, 2006.

[65] I. G. M. Brons, L. E. Smithers, M. W. B. Trotter et al., “Derivation of pluripotent epiblast stem cells from mammalian embryos,” Nature, vol. 448, no. 7150, pp. 191–195, 2007.

[66] P. J. Tesar, J. G. Chenoweth, F. A. Brook et al., “New cell lines from mouse epiblast share defining features with human embryonic stem cells,” Nature, vol. 448, no. 7150, pp. 196–199, 2007.

[67] A. M. Singh, D. Reynolds, T. Cliff et al., “Signaling network crosstalk in human pluripotent cells: a Smad2/3-regulated switch that controls the balance between self-renewal and differentiation,” Cell Stem Cell, vol. 10, no. 3, pp. 312–326, 2012.

[68] A. S. Bernardo, T. Faial, L. Gardner et al., “BRACHYURY and CDX2 mediate BMP-induced differentiation of human and mouse pluripotent stem cells into embryonic and extraembryonic lineages,” Cell Stem Cell, vol. 9, no. 2, pp. 144–155, 2011.

[69] D. S. Griffiths, J. Li, M. A. Dawson et al., “LIF-independent JAK signalling to chromatin in embryonic stem cells uncovered from an adult stem cell disease,” Nature Cell Biology, vol. 13, no. 1, pp. 13–21, 2011.

[70] C. James, V. Ugo, J.-P. Le Couédic et al., “A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera,” Nature, vol. 434, no. 7037, pp. 1144–1148, 2005.

[71] R. L. Levine, M. Wadleigh, J. Cools et al., “Activating mutation in the tyrosine kinase JAK2 in polycythaemia vera, essential thrombocythaemia, and myeloid metaplasia with myelofibrosis,” Cancer Cell, vol. 7, no. 4, pp. 387–397, 2005.

[72] F. Liu, X. Zhao, F. Perna et al., “JAK2V617F-mediated phosphorylation of PRMT5 downregulates its methyltransferase activity and promotes myeloproliferation,” Cancer Cell, vol. 19, no. 2, pp. 283–294, 2011.

[73] L. Rui, N. C. T. Emre, M. J. Kruhlak et al., “Cooperative epigenetic modulation by cancer amplicon genes,” Cancer Cell, vol. 18, no. 6, pp. 590–605, 2010.

[74] V. K. Tiwari, M. B. Stadler, C. Wirbelauer, R. Paro, D. Schübel, and C. Beisel, “A chromatin-modifying function of JNK during stem cell differentiation,” Nature Genetics, vol. 44, no. 1, pp. 94–100, 2012.

[75] W.-W. Tee, S. S. Shen, O. Oksuz, V. Narendra, and D. Reinberg, “Erk1/2 activity promotes chromatin features and RNA Pol II phosphorylation at developmental promoters in mouse ESCs,” Cell, vol. 156, no. 4, pp. 678–690, 2014.

[76] Y. Wang, S. Baskerville, A. Shenoy, J. E. Babiarz, L. Baehner, and R. Blöchl, “Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation,” Nature Genetics, vol. 40, no. 12, pp. 1478–1483, 2008.
C. Melton, R. L. Judson, and R. Blclloch, “Opposing microRNA families regulate self-renewal in mouse embryonic stem cells,” Nature, vol. 463, no. 7281, pp. 621–626, 2010.

O. Li, J. Li, and P. Dröge, “DNA architectural factor and proto-oncogene HMGA2 regulates key developmental genes in pluripotent human embryonic stem cells,” FEBS Letters, vol. 581, no. 18, pp. 3533–3537, 2007.

J. Nishino, I. Kim, K. Chada, and S. J. Morrison, “Hmga2 promotes neural stem cell self-renewal in young but not old mice by reducing p16Ink4a and p19Arf expression,” Cell, vol. 135, no. 2, pp. 227–239, 2008.

M. S. Kumar, E. Armenteros-Monterroso, P. East et al., “HMGA2 functions as a competing endogenous RNA to promote lung cancer progression,” Nature, vol. 505, no. 7482, pp. 212–217, 2014.

S. R. Viswanathan, J. T. Powers, W. Einhorn et al., “Lin28 promotes transformation and is associated with advanced human malignancies,” Nature Genetics, vol. 41, no. 7, pp. 843–848, 2009.

S. Dangi-Garimella, J. Yun, E. M. Eves et al., “Raf kinase inhibitory protein suppresses a metastasis signalling cascade involving LIN28 and let-7,” The EMBO Journal, vol. 28, no. 4, pp. 347–358, 2009.

A. Bertero, P. Madrigal, A. Galli et al., “Activin/Nodal signaling and NANOG orchestrate human embryonic stem cell fate decisions by controlling the H3K4me3 chromatin mark,” Genes & Development, vol. 29, no. 7, pp. 702–717, 2015.

K. Hayashi, S. M. C. D. S. Lopes, F. Tang, and M. A. Surani, “Dynamic equilibrium and heterogeneity of mouse pluripotent stem cells with distinct functional and epigenetic states,” Cell Stem Cell, vol. 3, no. 4, pp. 391–401, 2008.

M. Yamaji, J. Ueda, K. Hayashi et al., “PRDM14 ensures naive pluripotency through dual regulation of signaling and epigenetic pathways in mouse embryonic stem cells,” Cell Stem Cell, vol. 12, no. 3, pp. 368–382, 2013.

Z. S. Singer, J. Yong, J. Tischler et al., “Dynamic heterogeneity and DNA methylation in embryonic stem cells,” Molecular Cell, vol. 55, no. 2, pp. 319–331, 2014.

R. M. Kumar, P. Cahan, A. K. Shalek et al., “Deconstructing transcriptional heterogeneity in pluripotent stem cells,” Nature, vol. 516, no. 729, pp. 56–61, 2014.

G. Lahav, N. Rosenfeld, A. Sigal et al., “Dynamics of the p53-Mdm2 feedback loop in individual cells,” Nature Genetics, vol. 36, no. 2, pp. 147–150, 2004.

B. D. Macarthur, A. Sevilla, M. Lenz et al., “Nanog-dependent feedback loops regulate murine embryonic stem cell heterogeneity,” Nature Cell Biology, vol. 14, no. 11, pp. 1139–1147, 2012.

M. Fidalgo, F. Faiola, C.-F. Pereira et al., “Zfp281 mediates Nanog autorepression through recruitment of the NuRD complex and inhibits somatic cell reprogramming,” Proceedings of the National Academy of Sciences of the United States of America, vol. 109, no. 40, pp. 16202–16207, 2012.

N. Xu, T. Papagiannakopoulos, G. Pan, J. A. Thomson, and K. S. Kosik, “MicroRNA-145 regulates OCT4, Sox2, and KLF4 and represses pluripotency in human embryonic stem cells,” Cell, vol. 137, no. 4, pp. 647–658, 2009.

G. Pan, J. Li, Y. Zhou, H. Zheng, and D. Pei, “A negative feedback loop of transcription factors that controls stem cell pluripotency and self-renewal,” The FASEB Journal, vol. 20, no. 10, pp. 1730–1732, 2006.

A. Marson, S. S. Levine, M. F. Cole et al., “Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells,” Cell, vol. 134, no. 3, pp. 521–533, 2008.

H. Marks, T. Kalkan, R. Menafra et al., “The transcriptional and epigenomic foundations of ground state pluripotency,” Cell, vol. 149, no. 3, pp. 590–604, 2012.

T. Kunath, M. K. Saba-El-Leil, M. Almousailekh, J. Wray, S. M. Lemoche, and A. Smith, “FGF stimulation of the Erk1/2 signalling cascade triggers transition of pluripotent embryonic stem cells from self-renewal to lineage commitment,” Development, vol. 134, no. 16, pp. 2895–2902, 2007.

F. Yi, L. Pereira, J. A. Hoffman et al., “Opposing effects of Tcl3 and Tcl1 control Wnt stimulation of embryonic stem cell self-renewal,” Nature Cell Biology, vol. 13, no. 7, pp. 762–770, 2011.

D. ten Berge, D. Kurek, T. Blauwkkamp et al., “Embryonic stem cells require Wnt proteins to prevent differentiation to epiblast stem cells,” Nature Cell Biology, vol. 13, no. 9, pp. 1070–1075, 2011.

N. Lyashenko, M. Winter, D. Migliorini, T. Biechele, R. T. Moon, and C. Hartmann, “Differential requirement for the dual functions of β-catenin in embryonic stem cell self-renewal and germ layer formation,” Nature Cell Biology, vol. 13, no. 7, pp. 753–761, 2011.

J. E. Purvis and G. Lahav, “Encoding and decoding cellular information through signaling dynamics,” Cell, vol. 152, no. 5, pp. 945–956, 2013.

C. J. Marshall, “Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation,” Cell, vol. 80, no. 2, pp. 179–185, 1995.

S. Sasagawa, Y.-I. Ozaki, K. Fujita, and S. Kuroda, “Prediction and validation of the distinct dynamics of transient and sustained ERK activation,” Nature Cell Biology, vol. 7, no. 4, pp. 365–373, 2005.

S. D. M. Santos, P. J. Verveer, and P. I. H. Bastiaens, “Growth factor-induced MAPK network topology shapes Erk response determining PC-12 cell fate,” Nature Cell Biology, vol. 9, no. 3, pp. 324–330, 2007.

I. Amit, A. Citri, T. Shay et al., “A module of negative feedback regulators defines growth factor signaling,” Nature Genetics, vol. 39, no. 4, pp. 503–512, 2007.

T. Nakakuki, M. R. Birtwistle, Y. Saeki et al., “Ligand-specific c-Fos expression emerges from the spatiotemporal control of ErBb network dynamics,” Cell, vol. 141, no. 5, pp. 884–896, 2010.

R. Villasenor, H. Nonaka, P. D. Conte-Zerial, Y. Kalaidzidis, and M. Zerial, “Regulation of EGFR signal transduction by analogue-to-digital conversion in endosomes,” eLife, vol. 4, Article ID e06156, 2015.

D. Lakatos, E. D. Travis, K. E. Pierson, J. L. Vivian, and A. Czirok, “Autocrine FGF feedback can establish distinct states of Nanog expression in pluripotent stem cells: a computational analysis,” BMC Systems Biology, vol. 8, article 112, 2014.

F. Lanner, K. L. Lee, M. Sohl et al., “Heparan sulfation-dependent fibroblast growth factor signaling maintains embryonic stem cells primed for differentiation in a heterogeneous state,” Stem Cells, vol. 28, no. 2, pp. 191–200, 2010.

K. E. Galvin-Burgess, E. D. Travis, K. E. Pierson, and J. L. Vivian, “TGF-β-superfamily signaling regulates embryonic stem cell heterogeneity: self-renewal as a dynamic and regulated equilibrium,” Stem Cells, vol. 31, no. 1, pp. 48–58, 2013.

A. M. Singh, J. Chappell, R. Trost et al., “Cell-cycle control of developmentally regulated transcription factors accounts for heterogeneity in human pluripotent cells,” Stem Cell Reports, vol. 1, no. 6, pp. 532–544, 2013.
[110] R. Ho, B. Papp, J. A. Hoffman, B. J. Merrill, and K. Plath, “Stage-specific regulation of reprogramming to induced pluripotent stem cells by Wnt signaling and T cell factor proteins,” Cell Reports, vol. 3, no. 6, pp. 2113–2126, 2013.

[111] L. Marucci, E. Pedone, U. di Vicino, B. Sanuy-Escribano, M. Isalan, and M. P. Cosma, “Beta-catenin fluctuates in mouse ESCs and is essential for Nanog-mediated reprogramming of somatic cells to pluripotency,” Cell Reports, vol. 8, no. 5, pp. 1686–1696, 2014.

[112] F. Lluis, E. Pedone, S. Pepe, and M. P. Cosma, “Periodic activation of Wnt/β-catenin signaling enhances somatic cell reprogramming mediated by cell fusion,” Cell Stem Cell, vol. 3, no. 5, pp. 493–507, 2008.

[113] F. Aulicino, I. Theka, L. Ombrato, F. Lluis, and M. P. Cosma, “Temporal perturbation of the Wnt signaling pathway in the control of cell reprogramming is modulated by TCF1,” Stem Cell Reports, vol. 2, no. 5, pp. 707–720, 2014.

[114] B. Martynoga, D. Drechsel, and F. Guillemot, “Molecular control of neurogenesis: a view from the mammalian cerebral cortex,” Cold Spring Harbor Perspectives in Biology, vol. 4, no. 10, 2012.

[115] R. Kageyama, T. Ohtsuka, H. Shimojo, and I. Imayoshi, “Dynamic Notch signaling in neural progenitor cells and a revised view of lateral inhibition,” Nature Neuroscience, vol. 11, no. 11, pp. 1247–1251, 2008.

[116] M. Florio and W. B. Huttner, “Neural progenitors, neurogenesis and the evolution of the neocortex,” Development, vol. 141, no. 11, pp. 2182–2194, 2014.

[117] T. Ohtsuka, M. Ishibashi, G. Gradwohl, S. Nakanishi, F. Guillemot, and R. Kageyama, “Hes1 and Hes5 as Notch effecters in mammalian neuronal differentiation,” The EMBO Journal, vol. 18, no. 8, pp. 2196–2207, 1999.

[118] R. Kageyama, T. Ohtsuka, and T. Kobayashi, “The Hes gene family: repressors and oscillators that orchestrate embryogenesis,” Development, vol. 134, no. 7, pp. 1243–1251, 2007.

[119] I. Imayoshi and R. Kageyama, “Oscillatory control of bHLH factors in neural progenitors,” Trends in Neurosciences, vol. 37, no. 10, pp. 531–538, 2014.

[120] J. Hatakeyama, Y. Bessho, K. Katoh et al., “Hes genes regulate size, shape and histogenesis of the nervous system by control of the timing of neural stem cell differentiation,” Development, vol. 131, no. 22, pp. 5539–5550, 2004.

[121] D. S. Castro, B. Martynoga, C. Parras et al., “A novel function of the proneural factor Ascl1 in progenitor proliferation identified by genome-wide characterization of its targets,” Genes & Development, vol. 25, no. 9, pp. 930–945, 2011.

[122] Y. Sun, D. H. Meijer, J. A. Albert et al., “Phosphorylation state of Olig2 regulates proliferation of neural progenitors,” Neuron, vol. 69, no. 5, pp. 906–917, 2011.

[123] L. Cai, E. M. Morrow, and C. L. Cepko, “Misexpression of basic helix-loop-helix genes in the murine cerebral cortex affects cell fate choices and neuronal survival,” Development, vol. 127, no. 14, pp. 3021–3030, 2000.

[124] H. Hirata, S. Yoshiura, T. Ohtsuka et al., “Oscillatory expression of the bHLH factor Hes1 regulated by a negative feedback loop,” Science, vol. 298, no. 5594, pp. 840–843, 2002.

[125] H. Shimojo, T. Ohtsuka, and R. Kageyama, “Oscillations in Notch signaling regulate maintenance of neural progenitors,” Neuron, vol. 58, no. 1, pp. 52–64, 2008.

[126] I. Imayoshi, A. Isomura, Y. Harima et al., “Oscillatory control of factors determining multipotency and fate in mouse neural progenitors,” Science, vol. 342, no. 6163, pp. 1203–1208, 2013.

[127] D. J. Wong, H. Liu, T. W. Ridky, D. Cassarino, E. Segal, and H. Y. Chang, “Module map of stem cell genes guides creation of epithelial cancer stem cells,” Cell Stem Cell, vol. 2, no. 4, pp. 333–344, 2008.

[128] I. Ben-Porath, M. W. Thomson, V. J. Carey et al., “An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors,” Nature Genetics, vol. 40, no. 5, pp. 499–507, 2008.

[129] H. Mizuno, B. T. Spike, G. M. Wahl, and A. J. Levine, “Inactivation of p53 in breast cancers correlates with stem cell transcriptional signatures,” Proceedings of the National Academy of Sciences of the United States of America, vol. 107, no. 52, pp. 22745–22750, 2010.

[130] J. Kim, A. J. Woo, J. Chu et al., “A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs,” Cell, vol. 143, no. 2, pp. 313–324, 2010.