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Transcriptional regulation of neuronal differentiation: The epigenetic layer of complexity

Mary E. Hamby^{a,c}, Volkan Coskun^{a,b,c,*}, and Yi E. Sun^{a,b,c,*}

^aMental Retardation Research Center, David Geffen School of Medicine, University of California Los Angeles, USA

^bDepartment of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California Los Angeles, USA

^cDepartment of Psychiatry and Behavioral Sciences, David Geffen School of Medicine, University of California Los Angeles, USA

Abstract

The transcriptional programs of neural progenitor cells change dynamically during neurogenesis, a process regulated by both intrinsic and extrinsic factors. Although many of the transcription factors required for neuronal differentiation have long been identified, we are only at the brink of understanding how epigenetic mechanisms influence transcriptional activity and the accessibility of transcription factors to bind consensus *cis*-elements. Herein, we delineate the chief epigenetic modifications and the machinery responsible for these alterations. Further, we review the epigenetic modifications presently known to participate in the maintenance of the neural progenitor cell state and in the regulation of neuronal differentiation.

Keywords

miR-124; miR-9; Neurogenesis; REST; Chromatin remodeling; Histone modification; Acetylation; Methylation; miRNA; ncRNA; bHLH

1. Introduction

During development of the mammalian central nervous system (CNS), the generation of distinct cell types from neural precursors is carefully controlled and occurs successively with neurogenesis peaking first followed by the generation of astrocytes and then oligodendrocytes [1]. The correct timing and regulation of neurogenesis is crucial for proper CNS development and function as aberrant regulation can lead to several developmental disorders as well as tumorigenesis [2,3]. The transcriptional programs of neural stem cells (NSCs) and neuronal progenitor cells (NPCs) change dramatically at the time of neuronal differentiation in response to both intrinsic and extrinsic factors. Although many of the transcription factors required for neuronal differentiation have long been identified, we are only at the brink of understanding how factors other than the primary DNA sequence (i.e.,

*Corresponding authors. UCLA Medical School, Neuroscience Research Building, Suite 355, 635 Charles E. Young Dr. South, Los Angeles, CA 90095, USA. vcoskun@mednet.ucla.edu (V. Coskun), ysun@mednet.ucla.edu (Y.E. Sun).

epigenetic modifications) regulate the ability of these transcription factors to activate gene expression. Indeed, epigenetic alterations of the DNA and histones affect the overall structure and organization of chromatin and, as such, regulate the ability of transcription factors to access/bind to cognate *cis*-elements required to drive neuron-specific gene expression [4]. The literature supporting the integral role of epigenetic mechanisms in neurogenesis is vast and continually expanding and is the impetus for the herein review. We first introduce the concept of epigenetics, briefly describe the predominant mechanisms and the machinery responsible followed by a review of specific epigenetic modifications regulating neuronal differentiation.

2. Predominant epigenetic mechanisms

The term “epigenetics” has, in recent history, been defined as any meiotically or mitotically heritable change in gene function that cannot be ascribed to changes in the primary DNA sequence [4,5]. Although this is the definition used at present, it has become realized that a broader definition is necessary to encompass the dynamic flux in chromatin modifications that are not necessarily heritable but still result in changes in gene expression [5]. As such, the definition of epigenetics could be modified as “the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states,” as proposed by Bird [5]. Epigenetic modifications can be broadly divided into three major types: DNA methylation of CpG dinucleotides, covalent modifications of histone tails, and pre- and post-transcriptional modifications elicited by small non-coding RNAs (ncRNAs) [4,6,7]. Besides the direct effect of these modifications on gene transcription, epigenetic modifications can also act as platforms for chromatin-remodeling complexes, leading to more permanent changes in the chromatin state that can result in longer-term gene activation/silencing. Collectively, these changes result in the stabilization of transcriptional programs ultimately affecting the cellular phenotype [7,8].

2.1. DNA methylation

DNA methyltransferases (DNMTs) are responsible for methylating DNA on the 5 position of the pyrimidine ring of cytosine, which, in mammals, occurs almost exclusively at CpG dinucleotides. The methylation status of a given promoter is important in that DNA methylation in this region generally indicates that the gene is transcriptionally silent. There are two types of DNMTs: 1) *de novo* methyltransferases (e.g., DNMT3a, DNMT3b) that methylate non-methylated DNA and thus can establish DNA methylation patterns in a cell, and 2) maintenance methyltransferases (e.g., DNMT1) that recognize hemi-methylated DNA and methylate the single non-methylated strand during DNA replication [4,9].

2.2. Histone modifications

Another predominant epigenetic mechanism is the post-translational modification of histones. These modifications include acetylation, methylation, ubiquitination, sumoylation, ribosylation and phosphorylation of specific residues that typically, but not exclusively, reside in the amino terminal of histones [10]. One of the best described modifications is the acetylation of lysine residues on histone tails, a covalent modification mediated by histone acetyl-transferases (HATs). In general, this modification is thought to neutralize the

positively charged histones thereby weakening their tight interaction with negatively charged DNA. As a result, this loosens the chromatin structure enabling transcription factors to more readily access their cognate binding sites and generally promotes gene activation. Inversely, the deacetylation of histones, a modification mediated by histone deacetylases (HDACs), causes histone hypoacetylation which results in chromatin condensation and transcriptional silencing. In addition to the general loosening and tightening of chromatin due to alterations in the electrostatic interactions of histones and DNA, the reading of histone modifications by non-histone proteins (i.e., the histone code present) is integral in regulating function [11]. Chromatin-remodeling complexes have been shown to bind, via bromodomain proteins (e.g., Brg2), to specific acetylated lysines (e.g., acetylated H4K5, K8, K12 and K16) which can affect the transcriptional activity of the associated genes positively or negatively [11–17].

Another well-characterized histone modification is the methylation of lysines. Unlike the general global effects of altering the levels of DNA methylation and histone acetylation, the relative abundance of histone methylation does not distinguish transcriptionally competent or incompetent regions. Rather, the specific residue on a particular histone makes that distinction. Notably, methylation of lysine 4 on histone H3 (H3K4me) marks transcriptionally competent promoter regions whereas methylation of lysine 9 or lysine 27 on histone H3 (i.e., H3K9me or H3K27me) designates transcriptionally inactive regions [4]. Adding to the complexity of this modification, a single residue can be mono-, di-, or tri-methylated with each often resulting in divergent functional consequences [18]. There are several histone methyltransferases that are responsible for selectively methylating specific residues of specific histones. For example, the methyltransferase Suv39h1/2 has selectivity for methylating H3K9 [19] while mixed lineage leukemia (MLL) encodes a tri-methylase that is specific for H3K4 [20,21]. Histone demethylation occurs with similar specificity. For instance, lysine-specific histone demethylase 1 (LSD1) is a H3K4 mono- and di-demethylase [22] whereas UTX is a H3K27 demethylase [23–25]. For an exhaustive review of the recently discovered histone methyltransferases and demethylases and the histone residues they act upon please see [18].

2.3. Small non-coding RNAs (ncRNAs)

Small interfering RNAs (siRNAs) were first recognized as silencing gene expression at the post-transcriptional level by degrading mRNAs possessing a complementary sequence, a biological process known as post-transcriptional siRNA-mediated gene silencing (PTGS) [26]. Similarly, microRNAs (miRNAs), which are ~22 nucleotide (nt) ncRNAs, are involved in the post-transcriptional regulation of mRNA expression. More recently, siRNAs, miRNAs and small ncRNAs that have been described as double stranded small modulatory RNAs (smRNAs) have been shown to silence gene expression at the transcriptional level [transcriptional siRNA-mediated gene silencing (TGS)] [27–31]. For example, siRNAs can specify a target and, in a complex with argonaute – a protein required for PTGS – can bind to DNA to enable TGS [32,33]. This ncRNA/protein complex can facilitate long-term silencing of gene transcription through the recruitment of HDACs and DNMTs [34] which modify the chromatin and further recruit chromatin-remodeling complexes [26,35–39].

Ultimately, the aforementioned epigenetic mechanisms regulate many cellular processes including neuronal differentiation.

3. Epigenetic regulation of neuronal differentiation

The coincident upregulation of neurogenic factors and down-regulation of anti-neuronal factors instigates a sequence of events that leads to the terminal differentiation of neurons during development. Additionally, it is clear that epigenetic modifications play an integral role in both the maintenance of the NPC state and in neuronal differentiation. Indeed, in undifferentiated cells such as embryonic stem cells (ESCs) and NSCs, genes involved in cellular differentiation are thought to be “poised” for later expression through epigenetic modifications [40–42]. More specifically, in stem cells the majority of developmentally critical genes are “bivalent” in that they have both repressive- (e.g., H3K27me3) and activation-associated histone marks (e.g., H3K4me3) near the transcriptional start sites (or promoters) [42,43]. Upon differentiation, however, the genes integral to the differentiated cell type are typically enriched in activation-associated marks and lose the repressive marks previously present. In concert, the genes involved in the developmental regulation of other cell types generally retain the repressive marks but lose their activation-associated histone marks [40–42]. This illustrates the importance of understanding the role of specific epigenetic modifications in neuronal differentiation. Next the interaction of anti- and pro-neuronal factors with specific epigenetic modifications in NPCs and differentiated neurons will be reviewed.

3.1. Maintenance of the NPC state and suppression of neuronal differentiation

There is a vast literature delineating the role of anti-neuronal factors, namely the inhibitory basic helix loop helix (bHLH) transcription factors and repressor element 1-silencing transcription factor (REST), also known as neuron-restrictive silencing factor (NRSF), that are critical in the maintenance of the NPC state and in the prevention of precocious neurogenesis. Prior to neuronal differentiation, the inhibitory bHLH proteins, which include the Hes and Id families, are highly expressed in NPCs [44]. The Hes proteins impede neuronal differentiation through two predominant mechanisms: 1) via binding to their cognate *cis*-elements, N-boxes, thereby suppressing gene transcription and 2) via interacting with HDACs either directly [45] or indirectly through co-repressors such as Gro/TLE [46]. The interaction of Hes proteins with HDACs results in hypoacetylation of the histones associated with pro-neuronal promoters such as *Mash1* and *Ng2* and as a result subsequently stabilizes the inactive state of pro-neuronal genes preventing precocious neurogenesis from occurring [45,47]. Hes protein expression can be induced by Notch signaling but its expression is also autoregulated in a negative fashion [48]. For example, Hes1 can bind to N-boxes within its own promoter, inhibiting further Hes1 gene expression [48]. Another group of negative bHLH regulators, the Id family of proteins, lack the ability to bind to DNA; however, they can heterodimerize with pro-neural bHLH proteins causing a dominant negative effect by preventing bHLH factors from inducing pro-neuronal gene expression [49]. Interestingly, Id also modulates the expression/activity of Hes1. More specifically, Id can heterodimerize with Hes1 to prevent Hes1 from repressing its own gene expression [48]. These inhibitory bHLHs illustrate the highly regulated fashion of both the expression and

activity of pro-neuronal bHLH proteins, regulation which is vital in averting untimely neurogenesis and ensuring proper CNS development [48].

Another inhibitor protein critical in preventing precocious neuronal differentiation is REST (i.e., NRSF). REST binds to a 22 nt DNA binding sequence known as repressor element 1 (RE1), a site present within the promoters of several neuron-specific genes such as *Mash1* and *Bdnf*. REST acts in a complex with several co-repressors including CoREST, BRAF35, the histone demethylase BHC110/LSD1, Sin3b, the phosphatase SCP1, HDACs and the methyl-CpG binding protein 2 (MeCP2) in NPCs as well as in non-neuronal differentiated cells [50–52]. HDAC activity is required, at least in part, for the REST-mediated silencing of many neuron-specific genes in NPCs [52]. In support, there is a positive correlation between whether the RE1 sites present on specific neuronal promoters were physically associated with HDAC1/HDAC2 and whether those genes were upregulated in NPCs treated with an HDAC inhibitor [52]. Further, in an independent study, treatment of rat SVZ-derived neurospheres with HDAC inhibitors promoted neuronal differentiation [53]. Together, evidence suggests that HDACs play an important role in the induction of neuronal differentiation.

In addition to HDACs, REST also recruits chromatin-remodeling complexes such as the ATP-dependent chromatin-remodeling enzyme Brahma-related gene 1 (Brg1), a complex known to elicit robust changes in chromatin structure and is a subject matter that was recently reviewed [54]. In non-neuronal differentiated cells, it was shown that chromatin remodeling via Brg1 was required for REST-mediated silencing of certain genes [55] — although whether this is the case in undifferentiated NPCs has yet to be determined. In differentiated non-neuronal cells, in which neuronal genes need to be permanently silenced, there are also clear repressive epigenetic marks present surrounding RE1 sites [56]. But, contrary to that in non-neuronal differentiated cells, in mouse ESCs, although the REST repressive complex was found to be associated with certain promoters, the DNA was not methylated and the repressive mark histone H3K9 di-methylation (H3K9-di-me) was not observed [51]. The same study also found a marker of transcriptional competence H3K4me to be associated with pro-neural promoters in ESCs [51]. The lack of repressive marks in ESCs would make sense given that ESCs retain the potential to become neurons at a later time. However, the aforementioned study contrasts with another performed in a hippocampal neural progenitor cell line in which both repressive (i.e., histone H3K9-di-me) and active (i.e., histone H4 acetylation, H3K9 acetylation) epigenetic marks were found to be associated with REST-bound RE1 elements [52]. These marks were not only associated with REST but were actually due to the presence of REST as overexpression of dominant negative (DN)-REST led to an enrichment in the histone modifications associated with active gene transcription while decreasing those associated with gene silencing. Expression of DN-REST also enhanced neuronal differentiation as demonstrated by increased expression of TuJ1 and decreased expression of the NPC marker Nestin [52]. From this, it has been proposed that unlike the permanent silencing of neuronal genes required in non-neuronal cells, that in NPCs, the chromatin surrounding RE1 sites remains in a “poised” state ready for later activation upon neurogenesis [57]. Interestingly, the specific co-repressors present in the REST complex are heterogeneous and vary depending on the target

gene examined [51,52,58]. As such, it would be worthwhile to dissect the possible divergent functional roles of each distinct REST complex with regard to gene silencing.

3.2. NPC to neurogenic switch

Several switches in cellular machinery/gene programs are required in order for neurogenesis to proceed. These switches include changes in the composition of chromatin-remodeling complexes and in the pre-mRNA splicing programs, both participating in a global reorganization of gene transcriptional programs resulting in a downregulation of anti-neuronal factors and an upregulation of pro-neuronal factors. Indeed, the downregulation of REST is imperative in order to ensure proper and timely neurogenesis (Fig. 1) [59,60]. Depending on the experimental paradigm and system studied, downregulation of REST activity occurs by several means including via post-translational degradation, via retinoic acid-mediated inhibition of REST gene transcription, via HDAC activity and via ncRNA [29,30,51]. Concomitant with a decline in expression levels at the time of neurogenesis, REST disassociates from neuronal promoters such as *Mash1* [51]. Further, there appears to be a switch in composition of the REST complex associated with RE1 sites at this time. Neuron-restrictive silencing element (NRSE) dsRNA is induced at the time of neurogenesis and interacts with the REST complex. NRSE dsRNA causes a “switch” in the chromatin modifying factors present in the REST complex from repressive factors such as MeCP2 and HDAC to markers of activation (i.e., increased acetylated histones) [30]. This change in histone modifications associated with RE1 sites from repressive to that indicative of transcriptional activation correlates with an increase in pro-neuronal gene expression [29,30]. Moreover, NRSE dsRNA is required for neuronal differentiation as loss of NRSE dsRNA via use of antisense NRSE dsRNA prevents the neuronal differentiation of adult hippocampal stem cells [29,30]. Another change in REST complex composition occurs during the transition from NPC to neuron. The REST co-repressor BRAF35 is expressed in NPCs while iBRAF expression is enriched in mature neurons [20]. At the time of neurogenesis, iBRAF associates with the REST complex and recruits the histone methyltransferase MLL, which methylates histone H3K4, a modification associated with transcriptional activation near RE1 sites [20]. Further, ectopic expression of iBRAF results in a derepression of REST-silenced genes which results in enhanced expression of neuron-specific genes such as *NeuroD* and promotes neuronal differentiation [20]. The importance of the timely downregulation of REST prior to neuronal differentiation was recently illustrated [61]. Forced expression of REST in cerebellar Myc-expressing NPCs at the time of neurogenesis led to decreased neuronal differentiation by sustaining the progenitor-like state of NPCs. This resulted in the development of cerebellar tumors mirroring that of patients with medulloblastoma [61]. Hence, the down-regulation of REST at the time of neuronal differentiation is critical in proper development of the nervous system.

Another critical component in neuronal differentiation is the timely upregulation of pro-neuronal bHLH factors, which include Neurogenins (Ngn1 and 2), *NeuroD*, MASH1, *NeuroD*-related factor (NDRF) and *Nex* (also known as MATH2) [62–65]. Once a pro-neuronal bHLH factors is upregulated, it can act as heterodimers with the ubiquitously expressed E2A protein to bind to its cognate *cis*-element (i.e., E-boxes) triggering a pro-neuronal gene transcriptional program [66–68]. The temporal upregulation of pro-neuronal bHLHs

correlates with changes in epigenetic marks associated with pro-neural transcriptional start sites. For example, the epigenetic marks associated with the pro-neural bHLH promoter *Mash1* change drastically from high levels (in ESCs) to low levels (in neurons) of the repression-associated histone modification H3K27me3 with a simultaneous increase from ESCs to neurons in the activation-associated acetylation of H3K9 and the induction of *Mash1* mRNA expression [69]. Pro-neural bHLHs appear to enable neuronal differentiation partially through the SWI/SNF-like Brg/Brm-associated factor (BAF) chromatin-remodeling complex during neuronal differentiation [70,71]. First, neurogenic bHLH factors including *Ngn1* and *NeuroD* have been found to be physically associated with Brahma-related gene 1 (*Brg1*), an ATPase of the SWI/SNF-like BAF complex [72]. Further, *Brg1* is required for *Ngn* and *NeuroD*-mediated induction of neuronal differentiation in NPCs [72]. Finally, the chromatin-remodeling machinery actually changes just prior to neuronal specification. In fact, in mouse the composition of the BAF subunits changes from BAF45a and BAF53a in NPCs to BAF45b, BAF45c and BAF53b in differentiated neurons. This switch is important as ectopic expression of BAF45a and BAF53a prevented the proper neuronal differentiation of motor neurons in chicken [71].

Other ncRNAs besides NRSE dsRNA are important in facilitating neuronal differentiation. Notably, expression of the brain-specific miRNA, miR-124, is developmentally regulated with induction arising at the onset of neurogenesis and persisting in mature neurons (Fig. 1) [73–75]. As its expression profile might suggest, miR-124 does in fact promote neuronal differentiation [76–78]. The effect of miR-124 is specific for neurons as it is not expressed in astrocytes [75]. Further, when ectopically expressed in NPCs along with miR-9, (although expression of either alone resulted in no decrease in astrogliogenesis) an attenuation in astrogliogenesis (i.e., number of GFAP+ cells) and Stat3 activation transpired [77]. In turn, inhibition of miR-9 in NPCs decreases neurogenesis corresponding to a slight elevation in astrogliogenesis and enhanced activation of Stat3 with no further effect occurring when both miR-9 and miR-124 were inhibited [77]. At present, miR-124 can facilitate neuronal differentiation through two well-defined mechanisms. First, the miR-124-mediated enhancement of retinoic acid-induced neuronal differentiation was found to partially depend on the miR-124-triggered expression of PTBP2, an inhibitor of PTBP1 which splices out neuron-specific alternative exons [78]. Therefore, miR-124 is involved in eliciting the switch in pre-mRNA splicing patterns from non-neuronal to neuronal. Second, the targets of miR-124 are non-neuronal in nature and thus expression of miR-124 leads to the rapid degradation of several non-neuronal genes including members of the REST complex such as small C-terminal domain phosphatase 1 (SCP1) [57,76,79]. Interestingly, SCP1 is required for the REST-mediated silencing of neuronal genes in differentiated non-neuronal cells [80]. Overexpression of miR-124 in the chick neural tube elicits the upregulation of neurogenic *Ngn2* and *NeuroD* expression and, even more importantly, causes precocious neuronal differentiation [76]. Indeed, this enhancement in neurogenesis could be blocked by overexpression of SCP1 [76]. Overall, the simultaneous upregulation of pro-neural miR-124 and downregulation of members of the REST complex allows for a timely transformation of a NPC to a differentiated neuron (Fig. 1; [57]). Importantly, since in NPCs miR-124 expression is inhibited by REST [57] through the three RE1 sites present on its promoter, once miR-124 is expressed its ability to downregulate non-neuronal genes – particularly

those participating in the REST complex – should greatly facilitate a swift transition from the neural progenitor-like state of the cell to differentiated neuron [57,76].

It is likely that other miRNAs are involved in neurogenesis of specific subtypes of neurons. Mutant mice that lack Dicer specifically in olfactory progenitor cells (via conditional deletion in *Foxg1*-expressing cells) lack terminal differentiation of olfactory neurons [81]. Interestingly, this phenotype could be recapitulated by knock down of the miR-200 family of miRNAs in zebrafish suggesting that this family of miRNAs is required for terminal differentiation of olfactory neurons [81]. In the eye of *Drosophila*, miR-7 appears to promote photoreceptor differentiation [82]. Overexpression of miR-133b in NPCs reduced the number of tyrosine hydroxylase (TH)-positive cells with no change in overall numbers of Tuj1-positive cells (i.e., neurons) while inhibition of miR-133b expression upregulated the expression of the dopamine neuronal markers such as TH suggestive of a role for miR-133 in the differentiation of dopaminergic neurons specifically [83].

3.3. Neuro- to gliogenic switch

Pro-neuronal bHLHs also play a role in regulating the timing of the neuro- to gliogenic switch. In support, ectopic expression of *Ngn1* in cortical NPCs prevented the ability of ciliary neurotrophic factor (CNTF) to induce astrogliogenesis [62]. This effect was not dependent on the ability of *Ngn1* to induce neurogenesis but, rather, was due to the sequestration of a transcriptional co-activator complex, namely CBP/p300 (which has intrinsic HAT activity) and *Smad1*, thus hindering the interaction of this complex with *Stat3*, a process known to be important in facilitating astrogliogenesis [49,62,84,85]. Thus, bHLH factors also participate in regulating the critical timing of the neurogenic to gliogenic switch allowing for the proper production of neurons at the time of neurogenesis [62,86].

Another process essential to the timing and proper generation of neurons is the methylation status of the DNA. Prior to astrogliogenesis, the methyl-CpG binding protein MeCP2 is bound to the *Gfap*, *Stat1*, and *S100B* promoters but not *STAT3*. However, during astrogliogenesis, the association of MeCP2 and *STAT3* to these promoters is inverse to that in NPCs. During the neurogenic to gliogenic switch, multiple CpGs including a site on the *STAT*-binding element of the *Gfap* promoter become demethylated along with several CpGs present on the *Stat1* and *S100β* promoters [87–89]. Further, when global DNA hypomethylation is present in NPCs derived from mice conditionally lacking DNMT1, precocious astrogliogenesis occurs at the expense of neurogenesis [87]. The histone modifications associated with the *Gfap* and *Stat1* promoters also shift from bivalent (i.e., both H3K9-di-me and H3K4-di/tri-me) or mostly repressive (i.e., H3K9-di-me) marks, in NPCs to those indicating transcriptional activity (H3K4-di/tri-me) during the time of astrogliogenesis [87]. Collectively, it is evident that the epigenetic landscape of a cell (e.g., DNA methylation status) plays a fundamental role in governing whether or not the neurogenic or gliogenic switch occurs in a proper and timely manner.

4. Future directions

Future exploitation of recent genome-wide techniques should enable further clarification of the role of epigenetic modifications and transcriptional programs involved in neuronal

differentiation. Recent technological advances have demonstrated the power of using genome-wide analyses, for example with the use of ChIP-chip and now ChIP-seq [43]. Using these technologies, comparisons of the epigenetic landscape between NPCs and differentiated neurons, and even of different neuronal subtypes, can be made. Recently a global analysis (i.e., serial analysis of chromatin occupancy) of REST sites was performed which identified many RE1 sites that were previously unknown [90]. From this, many more neuron-specific genes were discovered that have the potential to be regulated by REST and thus will need to be studied in more detail [90]. Ultrahigh-resolution tiling microarrays are also a high-throughput method that can be used to decipher the epigenetic landscapes of both genic and intergenic regions, which often encode ncRNAs [91]. Hence, employment of this methodology should aid in the identification of previously unknown genes and ncRNAs involved in neuronal differentiation. In addition to the advantage of pure discovery of these genome-wide approaches, use of these approaches in conjunction with a time course spanning the developmental course from NPC to neuron may help to tease out the order of transcriptional events and how epigenetic medications modulate this process. Clearly, delineation of the pathways underlying transcriptional programs and epigenome-wide modifications will be beneficial in understanding the entire intricate process of neuronal differentiation.

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Fig. 1.

Key events in the NPC to neuron transition. During neurogenesis, many events in the cell are orchestrated to ensure proper neuronal differentiation. Although there are a number of players, miR-124 expression and the downregulation of REST play key roles in globally decreasing expression of non-neuronal genes while simultaneously enhancing neuron-specific gene expression. When REST expression is downregulated, this enables the derepression of numerous pro-neuronal genes allowing for pro-neural bHLHs to instigate the further induction of neuronal genes. Moreover, dismissal of REST from the RE1 sites on *miR-124* eradicates the silencing effect that REST has on *miR-124* [57], allowing for induction of miR-124 expression. Once miR-124 is induced, it can then degrade its numerous non-neuronal targets [57,76]. Collectively, the downregulation of REST and upregulation of miRNA provide a swift transition from NPC to neuron.