

Chapter 2

Understanding Epigenetic Memory is the Key to Successful Reprogramming

Abstract Molecular biologists have developed powerful tools for measuring and/or describing a cell's epigenome, including: bisulfite-sequencing, chromatin-immunoprecipitation (ChIP), and comprehensive high-throughput arrays for relative methylation (CHARM). Over the last few years, a body of work has emerged using such techniques to map the entire epigenetic landscape of ESCs, iPSCs, and terminally differentiated cells. In this way the underlying mechanisms of differentiation, as well as the effects of artificial de-, re-, and trans-differentiation can be understood by comparing important differences in their epigenetic states.

Keywords Epigenetics • DNA methylation • Histone modification • Noncoding RNA • Epigenetic maps • CNS development

2.1 Epigenetic Influences on Gene Expression

Long term continuous TF-based reprogramming of iPSCs is required to achieve sufficient numerical efficiency as well as qualitative pluripotency. This suggests that TFs on their own face difficulties in rearranging epigenetic marks. This is somewhat logical, as most TFs would employ indirect mechanisms to adjust epigenetic states. Further, current repressive marks might have to be overcome for TFs to bind properly.

Different studies have shown that direct control of epigenetic mechanisms can improve reprogramming speed, efficiency, and quality. However, before discussing epigenetic factors that might aid reprogramming, a short overview of epigenetic mechanisms will be presented.

2.1.1 DNA Methylation

The most common form of epigenetic DNA modification is methylation of cytosine. Within mammals, and more prominently in humans, cytosine-guanine dinucleotide sequences tend to be grouped together in so-called CpG islands around gene promoters. Depending on frequency, this allows genes to be identified as having high (HCP) or low (LCP) CpG density promoters. Methyl groups are added to the cytosine residues of CpGs by various DNA methyltransferases (Dnmts). For example, Dnmt1 provides continuity of methylation patterns as a *maintenance* methyltransferase, while Dnmt3a and Dnmt3b establish new methyl groups as *de novo* methyltransferases. Once placed, methyl groups at promoter sites may prevent binding of transcription factors and so gene expression. They may also attract and assist repression complexes, which also prevent gene expression. This means that in general, methylation of DNA is a repressive epigenetic mark. Loss of Dnmts can cause severe deformities and death, indicating that proper epigenetic DNA methylation patterns are essential to development and health. One study determined that reaching a state of terminal differentiation requires Dnmt1 in specific, clarifying its importance to establishing cell identity (Jackson et al. 2004).

2.1.2 Histone Modification

Amino acid residues making up the tails of histone proteins are targets for modification. Such modifications include methylation, acetylation, phosphorylation, and ubiquitination. The specific effects of histone modification are dependent on position/type of amino acid, and type/degree of modification. Research is slowly building a histone code to relate modifications to epigenetic effects. This is a very large field of research and for practical reasons will not be discussed in detail. The most common, and therefore well researched, modifications are methylation and acetylation. Methyl groups are added by histone methyl-transferases (HMTs), while acetyl groups are added by histone acetyl-transferases (HATs). Histone deacetylation and demethylation is performed by HDACs and HDMs, respectively. There are also histone-remodeling complexes such as SWI/SNF capable of moving, eliminating, or swapping out specific histones which *de facto* removes present histone coding.

Acetyl groups are thought to open chromatin structure directly by introduction of a negative charge, while methyl groups tighten chromatin structure, directly or through interaction with protein complexes capable of structural/functional changes. Thus acetylation tends to be an activation mark, while methylation tends to be a repressive mark. It must be remembered though; active and repressive effects depend on many factors. For example, while tri-methylation of the 27th lysine residue of histone protein 3 (H3K27me3) is a repressive mark, trimethylation of the

4th lysine of the same histone protein (H3K4me3) is an active mark. It is also possible to have more than one kind of modification on a single histone protein, and across histones of different nucleosomes. The polycomb group (PcG) of proteins contains important repressive complexes for gene regulation, particularly the HMT polycomb repressive complex (PRC)-2 that methylates H3K27, and HUT PRC1 that ubiquitinates H2AK119. Problems with PRC2, including loss of subunits, have been shown to cause severe defects in embryonic development (Surface et al. 2010). In contrast, the Trithorax group (TrxG) of proteins includes HMTs that are equally important in placing active H3K4me3 marks. H3K4me3 is highly associated with DNA hypomethylation and upregulated gene expression. Interestingly, loss of both H3K27 and H3K4 trimethylation is correlated with DNA hypermethylation (Meissner et al. 2008). This indicates that epigenetic marking of histones, as DNA methylation, is important to proper development and health.

2.1.3 Noncoding RNA

There are numerous DNA sequences which do not code for any cellular protein, but may be expressed as non-protein coding RNA (ncRNA). These can reside between genes or within them as introns, and may take many forms. Two key forms have been indicated as playing roles in the epigenetics of pluripotency and cell identity: long noncoding RNA (lncRNA) and miRNA. While not always classed as true epigenetic factors, as they require sequence expression that is itself regulated by histone and DNA modifications, they play epigenetic-like roles in altering gene expression profiles and in some cases are integral parts of the epigenetic systems that set and maintain repressive/active patterns.

lncRNA refers to expressed, non-protein coding sequences that are longer than 200 nt. These are estimated to constitute many thousands, perhaps hundreds of thousands, of expressed sequences found in mammals (Birney et al. 2007; Kapranov et al. 2007). While they may be processed in many ways, including as precursors to miRNAs, their role in conjunction with epigenetic mechanisms is becoming clear. Two well known lncRNAs with developmental significance are X-inactive transcript (Xist), which silences X-chromosomes by coating the one from which it was transcribed (cis-acting), and HOX antisense intergenic RNA (HOTAIR) which acts to silence genes on another chromosome (trans-acting). MiRNAs originate as long RNA sequences that undergo enzymatic processing inside the nucleus (by DGCR8/Drosha) and outside (by TRBP/Dicer), leaving short (~22 nt) segments which are taken up by RNA-induced silencing complex (RISC) (Mallanna and Rizzino 2010). RISC then uses miRNAs to target DNA for transcriptional blocking or degradation. MiRNAs are recognized as potentially powerful regulators of gene expression and cell identity. For example, the transgene LIN28 used in iPSC protocols is an RNA binding protein that appears to exert its effect by reducing levels of the miRNA Let-7. Experimentally generated Dicer-null cells cannot create mature miRNA, and developmental abnormalities are

observed (Li and Jin 2010; Mallanna and Rizzino 2010). This shows that miRNAs, as other epigenetic factors, are necessary for proper development and health.

2.1.4 Common Epigenetic Traits

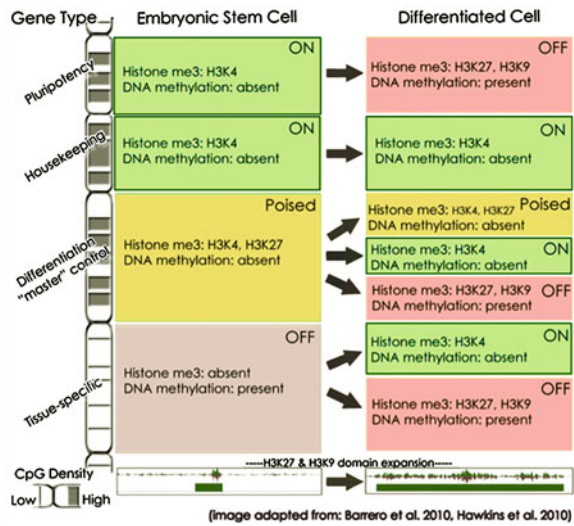
Regardless of cell type, the epigenetic mark H3K36me3 is enriched across transcribed regions, following the active H3K4me3 mark (Mikkelsen et al. 2007). This makes sense given its relation to RNA polymerase II transcription, specifically elongation. Its presence was much less prominent in bivalent genes, which contain mixed active and repressive marks, and little overlap was seen between H3K36me3 and repressive H3K27me3 marks. It has been argued that these properties make H3K36me3 a useful epigenomic mapping target for expressed sequences, as “transcriptional units”, particularly ncRNAs. It was also found that overlapping areas of H3K4me3 and H3K9me3 indicate imprinting regions in ESCs. Another mapping experiment, found that H3K27ac occurs in “peaks” like the active H3K4me3 mark, suggesting a similarity between active marks and resulting chromatin structure regardless of acetylation or methylation (Hawkins et al. 2010). Together these point to common epigenetic signals that could be exploited to determine current gene or chromatin status within a cell.

2.2 Epigenetic Control in De-, Re-, and Trans-Differentiation

Creation of iPSCs requires the rearrangement of existing repressive and activating marks, across the entire genome, back to those held by ESCs. The change of any mark could be viewed as a single de-differentiating event, with success affected by the stability of the mark, as well as ongoing mechanisms that work to sustain the mark. Re- and trans-differentiation constitute the same type of process as de-differentiation, being the rearrangement of required marks to adjust chromatin structure and so gene expression of a desired cell type. Therefore, epigenetic maps of iPSCs, and conclusions based from them, will be used to represent all reprogramming methods.

The most significant problem in any reprogramming method will be the issue of epigenetic accuracy. There are basically three states a gene can be in: *off*, *on*, and *poised* (Fig. 2.1). It is that third category that makes things less than straightforward, as reprogramming cannot be as simple as removing all repressive or activating marks. It is somewhat inconvenient that the most important genes for proper development need to be reset to *poised*, with an active equilibrium of repressive and activating marks (Hawkins et al. 2010). It is also possible for the process of reprogramming to set entirely new marks, unrelated to desired status. Thus while a reprogrammed cell might show adequate gene expression levels throughout most of its genome, indicating success, a bivalent gene not set properly can generate

Fig. 2.1 Epigenomic map of embryonic stem and differentiated cells. Genomes are broken into regions with low/high CpG content at gene promoters, and further by gene types. Changes from pluripotent ESC state to full differentiation are described. At the *bottom*, expansion of repressive domains (ex. H3K27me3) during differentiation is shown. iPSC reprogramming must reset all marks properly



functional problems later when environmental conditions change. This is supported by a report where aberrant silencing of just one gene cluster affected proper cell function (Stadtfield et al. 2010). Another study of ESC and iPSC methylomes showed that developmental genes of iPSCs were in a methylated state somewhere between ESCs and fibroblasts. Supporting theories that reprogramming can actively place improper epigenetic signatures, aberrant marks unrelated to ESCs or source cells were detected (Doi et al. 2009). Also, significant repressive marks naturally gained during differentiation, like H3K9me3, are removed inefficiently or even added to during de-differentiation (Mikkelsen et al. 2007). As it is, the expansion of H3K9 and H3K27me3 domains during differentiation could potentially hinder full reprogramming due to retention of some portion of the expanded domains (Hawkins et al. 2010). A recent report with detailed (single base pair resolution) epigenetic comparisons of ESCs, iPSCs, and somatic cells, showed that consistent aberrant marks were generally localized to centromeres and telomeres (Lister et al. 2011). This suggests another, in this case mechanical, problem for complete reprogramming in selected areas of chromosomes, which can affect various genes. As with other studies, Lister et al. (2011) found instances of both incomplete reprogramming and newly imposed improper epigenetic marks. Re-differentiation bias of iPSCs has also been investigated from an epigenetic standpoint (Kim et al. 2010). This revealed that DNA methylation signatures from the original cell source remained in iPSCs. These signatures appeared capable of hindering iPSC re-differentiation to other cell types, while positively affecting re-differentiation to initial cell lineage. Lister et al. (2011) re-differentiated iPSC lines and found that aberrant marks were passed on to daughter cells, which could affect cell fate decisions as well as function (though this was not proven).

MiRNAs can also be used to distinguish between cell types. 49 human cell lines were examined to compare the expression levels of 330 miRNAs (466 in some cases) across hESCs, differentiated cells, hiPSCs, and cancer cells (Neveu et al. 2010). Contrary to the authors' expectations, statistical analysis generated four clusters of cell types. In addition to healthy differentiated cells and cancer cells, there were two different clusters which both contained hESCs and hiPSCs. The grouping of hESCs and hiPSCs together suggests that reprogramming was largely sufficient to regenerate ESC miRNA levels. This is consistent with other research that showed few differences in miRNA levels between hESCs and hiPSCs (Chin et al. 2009; Stadtfeld et al. 2010). Of interest was that one cluster of hESCs and hiPSCs differed from the other, based on some of the same miRNA profiling criteria that separate healthy cells from cancer cells (Neveu et al. 2010). These were connected to the p53 network that regulates cell growth and division, as well as apoptosis. It was advanced that these two pluripotent clusters could create subdivisions of pluripotent cells, similar to naïve versus primed, based on the integrity of their p53 network. A connection was also made between the TF cMyc, which regulates cell growth and division, and iPSC clustering with cancer cells. In their analysis, down regulation of cMyc resulted in a categorical shift away from cancer, suggesting that iPSCs that clustered with cancer might have higher levels of c-Myc (Neveu et al. 2010). As c-Myc is down regulated in later stages of reprogramming, where Oct4 becomes more important for establishing pluripotency, high levels of c-Myc in iPSCs could be an indicator that they are still in an early stage and not yet fully pluripotent. Thus miRNA profiles with similarities to cancer cells could be used as a marker of incomplete reprogramming in iPSCs (and ESCs which have moved toward "primed" state, or gained cancer characteristics).

2.3 Using Epigenetics to Aid Reprogramming

Huangfu et al. (2008a) were the first to test the use of epigenetic inhibitors on induction of pluripotency. Given the repressive nature of Dnmts and HDACs, it was theorized that their inhibition might aid reprogramming by easing repression and so opening chromatin to TFs. During induction of pluripotency using four TFs (OSKM), 5'-azacytidine (5'-azaC) was used to block Dnmt processing, while suberoylanilidehydroxamide acid (SAHA), trichostatin A (TSA), and VPA were used to block HDACs. All improved efficiency to some degree, however 5'-azac and VPA proved the most useful. VPA in particular had dramatic results, improving efficiency 50–100 fold (to above 2%) while reducing required reprogramming time from 30 days down to 2 weeks and successfully replacing the TF c-Myc. The same research group later reported VPA's ability to replace both Klf4 and c-Myc, reducing needed TFs further to just Oct4 and Sox2 (Huangfu et al. 2008b). Another paper reviewing small molecules used to improve reprogramming, reported three additional inhibitors: BIX-01294 that targets G9a HMTase, RG108 targeting Dnmts, and Parnate that targets lysine-specific HDMTs (Li and Ding 2010).

Improvements in reprogramming duration and efficiency due to epigenetic inhibitors were detailed in a recent review (Wang et al. 2010). Of all inhibitors examined, BIX and VPA appear to be the most powerful. Another lab tried to create a TF-free iPSC reprogramming method, which was entirely epigenetic (Han et al. 2010). The concept was to use ESC extracts, in combination with Dnmt and HDAC inhibitors. In this case 5'-azaC and TSA were used to treat somatic cells prior to transfer of extract. While full reprogramming was not accomplished, partial pluripotency was achieved, with cells apparently re-differentiating successfully. Dnmt and HDAC inhibitors were reported to improve extract performance. An earlier attempt, using only 5'-azaC and TSA on neurosphere cells, provided similar results (Ruau et al. 2008). There, histone acetylation and DNA demethylation were found to be promiscuous with respect to chromosomes, however genes associated with pluripotency were upregulated (ex. Oct4, Sox2, Klf4, Nanog, and cMyc). More interesting, while full pluripotency was not induced, neurosphere cells gained sufficient plasticity to re-differentiate to hematopoietic cells.

Given the greater efficiency (~45%), speed (1–2 days), and cell-division independence of SCNT and cell fusion reprogramming compared to TF-based methods (Han et al. 2010; Markoulaki et al. 2008), it is clear that ESCs and oocytes contain active epigenetic reprogramming components. One group screened extracts from pluripotent mouse ESCs and found two components, Brg1 and Baf155, associated with reactivation of Oct4 (Singhal et al. 2010). Both components were identified as subunits of the SWI/SNF complex, involved with nucleosome rearrangement as well as gene regulation via collaboration with epigenetic repressors (e. DNMT3a/b). When included as transgenes along with OSKM, or OSK, the components Brg1 and Baf155 improved induction efficiency (up to 12 fold). Their method of epigenetic action was also investigated. Euchromatin was increased, generally through enrichment of active marks such as H4Kme3 and H3K9ac. It was suggested that these subunits may have interfered with somatic SWI/SNF complexes and/or formed other complexes capable of enhancing reprogramming.

Two separate studies have recently proposed activation-induced cytidine deaminase (AID) as the active DNA demethylation component in mammals. Rather than direct demethylation of CpGs, AID would bind to 5-methylcytosine, followed by deamination of 5 mC to a thymine residue. The resulting T-G mismatch would then be repaired (i.e. return of Cytosine) by the base excision repair pathway (BER). One study used mammalian primordial germ cells (PGCs), which normally undergo DNA demethylation as part of their developmental process (Popp et al. 2010). AID deficient PGCs showed greater global methylation (3x) as compared to wild type, implicating their role in the demethylation process. It was suggested that this could be a mechanism for preventing transmission of epigenetic markings to offspring. The other study focused more specifically on reprogramming factors within ESCs. The authors took a novel approach and produced interspecies heterokaryons, specifically fusing mESCs with human fibroblasts, then knocked down AID using small interfering RNA (siRNA) (Bhutani et al. 2010).

Under normal conditions mouse ESCs were capable of inducing pluripotency in human fibroblasts quickly (1d, no cell division), and efficiently (70%). On transient knockdown of AID, induction of pluripotency (specifically expression of Oct4 and Nanog) was greatly inhibited (80%). This was true even at 35% knockdown of AID, indicating great sensitivity to its absence, and arguing for its necessity in normal mammalian gene regulation. In support of this, CpG areas of Oct4 and Nanog promoters in knockdowns were found to retain heavy methylation. When human AID was over expressed, prior to knockdown of mouse AID, demethylation activity was rescued to some degree. Nanog showed expected demethylation of promoters and upregulated gene expression. Oct4 exhibited only partial recovery to normal levels. Further, using ChIP (chromatin immunoprecipitation), the authors found that AID binds to heavily methylated promoters (specifically Oct4 and Nanog) in fibroblasts (where DNA demethylation is required) while not at hypomethylated promoters in mESCs. Taken together, both studies paint a very strong picture of AID being one of the key natural epigenetic reprogramming mechanisms, likely required for fast and efficient iPSC methods. However it remains to be determined what the exact follow up step to deamination involves, and if there are additional players required for both activating AID or repair of T-G mismatch (Agarwal and Daley 2010; Deng 2010).

As a note of caution, another study attempting to define the demethylation components in PGCs, similar to Popp et al. (2010), came to a different conclusion about the importance of AID (Hajkova et al. 2010). As zygotes were used in place of PGCs, their results were just as pertinent to the findings of Bhutani et al. (2010). This group tracked methylation changes in the maternal and paternal pronucleus, and its relation to BER (including knockdown using inhibitors of BER). They determined that deamination was not required, and that single strand DNA breaks were sufficient to activate BER, with resulting DNA demethylation. AID might have played a role, but was dismissed based on not finding evidence for it within the zygote at that stage. Curiously the authors cite Popp et al. (2010) in support, stating that AID in that report was shown to have little effect in PGCs, but that appears inconsistent with the written conclusions of Popp et al.. Of course it is possible that AID does not play a role in PGCs or primitive zygotes, but does so in later ESC tissue. Finally, both Popp et al. and Hajkova et al. propose Tet 5mC-hydroxylases as another potential mechanism for DNA demethylation. Clearly, further research is required to sort these possibilities out, but it does appear that identification of active epigenetic mechanisms is approaching.

NcRNA might also play a role in enhancing reprogramming efficiency and speed. Since ncRNA is a vast field, and arguably not a true epigenetic mechanism, research into using it for reprogramming will not be covered in as much detail. Two reviews of using miRNAs for reprogramming cell identity cite active research as well as its potential (Mallanna and Rizzino 2010; Sun et al. 2010). The main concept outlined by both is that miRNAs may be used (or inhibited) to target specific pathways, and downstream effectors, to create desired effects while limiting unintended problems. Both name p53 as an example. While the p53 network can block full iPSC reprogramming, knocking out p53 leads to problems.

However, p53's hindrance to reprogramming appears to be limited to its activation of miRNA-145, which blocks the key TFs Oct4, Sox2, Klf4, and cMyc. Thus one could target miR-145 for inhibition, and potentially enhance reprogramming, while retaining p53's other desired effects. This indicates that abundant miRNAs expression in ESCs and/or inhibition of miRNAs in the source somatic cells, could aid reprogramming.

As mentioned in [Chap. 1](#), miRNAs have moved beyond assisting standard TF-based reprogramming to become the method itself (Anokye-Danso et al. [2011](#); Miyoshi et al. [2011](#)). The approach of Anokye-Danso et al. ([2011](#)) to fully reprogram mouse and human fibroblasts to pluripotency using expression of the miR-302/367 cluster was more efficient and faster than the common OSKM TF method. Intriguingly this study also showed the potential utility and limitation of epigenetic factors in assisting reprogramming. While VPA's strengths have been noted earlier, this group found it was only useful for reprogramming mouse fibroblasts. This was argued to be due to VPA's targeting of HDAC2 proteins. Mice have relatively higher levels of HDAC2 and so require VPA for miR-302/367 reprogramming, while humans already produce low levels of HDAC2 which are unaffected by VPA. This means that epigenetic assistance may have to be species as well as cell specific.

2.4 Epigenetics Affecting Neural Cell Fate

After reprogramming to pluripotency, epigenetics can aid differentiation to neural cell lineages. The developmental pathway from ESC to NSC to neuron or glia is the subject of much research (Juliandi et al. [2010](#)). A genome-wide analysis of DNA methylation in NSCs, revealed that promoter regions of astrocytic genes are progressively demethylated from mid to late stages of development ([Fig. 2.2](#)) (Hatada et al. [2008](#)). This holds true for the astrocyte gene GFAP whose promoter has been shown to exhibit hypermethylation in mESCs, only becoming hypomethylated on reaching a mature NSC state (Shimozaki et al. [2005](#); Takizawa et al. [2001](#)). This is consistent with the general concepts already described, with DNA methylation blocking expression (in this case of astrocytes specific genes) by preventing the binding of transcription machinery. In addition, methylation of exons within GFAP gene sequences has been observed (Setoguchi et al. [2006](#)). In this case, methyl-CpG-binding domain proteins (MBDs) can latch on to the methylated exon and prevent transcription, even if the promoter of the gene is unmethylated. This allows another level of gene control and so greater flexibility. Both active and passive mechanisms have been suggested for DNA demethylation, and so activation of astrocyte genes. An active method would involve removing methylated cytosines, perhaps by the base excision repair mechanism (Ma et al. [2009](#)). Passive demethylation would come from blocking Dnmt1 maintenance activity, for example by nuclear factor 1A (NF1A) that has been reported to block Dnmt1 and allow astrocyte production (Namihiro et al. [2009](#)).

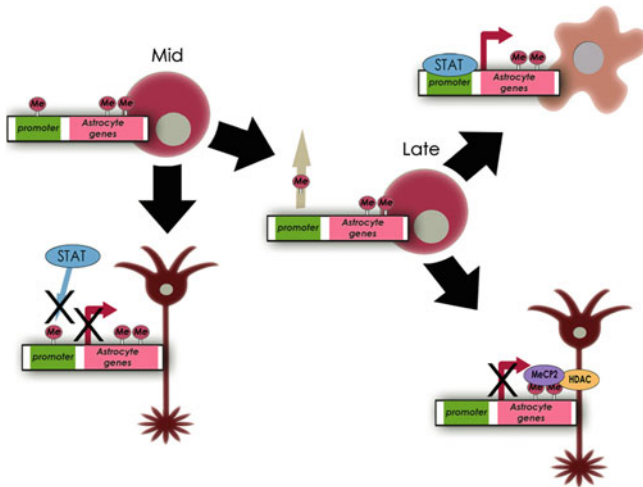


Fig. 2.2 DNA methylation regulates CNS development. Two paths of gene regulation by DNA methylation are shown. For mid-gestational NSCs, methylated promoters block transcriptional machinery preventing astrocytic gene expression. In late-gestational NSCs, methyl groups are absent from promoters but methyl CpG binding proteins may form complexes on methyl groups located within astrocyte gene sequences blocking transcription

Histone modifications play various roles during CNS development. Histone acetylation is largely associated with the opening of chromatin, and for CNS development plays a large role for neuronal genes. HDACs that remove such marks are known to prevent formation of neurons during development, and conversely HDAC inhibitors such as VPA have been shown to promote neuron production over glia in vitro (Hsieh et al. 2004). Specific HDMTs resolve bivalency, committing mESCs to NSC fates (Burgold et al. 2008). Later in development, H3K27 methylation of proneuronal genes like Neurogenin1 work to promote astrocyte production, in that particular case by inhibiting an astrocyte gene suppressor (Hirabayashi et al. 2009; Sun et al. 2001).

lncRNAs guide CNS development as well. The role of lncRNAs in regulating TFs may be particularly important for development and function of the CNS (Qureshi et al. 2010). An lncRNA known as Sox2ot was reported as being expressed in areas of active neurogenesis in adults, and that it might also regulate Sox2 TF levels during development (Amaral et al. 2009; Mercer et al. 2008). Finally, in vitro differentiation of NSCs into the oligodendritic cell lineage was improved with forced expression of lncRNA Nkx2.2AS (Tochitani and Hayashizaki 2008). Specific miRNAs are critical for early stages of neuronal lineage commitment and neural tube closure (ex. let-7), as well as regulating the proliferation and commitment of NSCs and NPCs to neural or glial fates (ex. miR-9, miR-23, and miR-125(b)) (Li and Jin 2010). MiR-124a enables RISC to degrade glia transcripts, blocking commitment to non-neuronal fates (Conaco et al. 2006). Developing cells from ESCs to NSCs express a protein (NRSF; neural-restrictive

silencer factor, also known as REST) that down regulates both neuronal genes and miR-124a, preventing premature generation of neurons. As development continues another miRNA, miR-9, may be expressed targeting NRSF. This lifts repression of neuronal genes and miR-124a, the latter suppressing non-neuronal transcripts, thereby enhancing neuronal commitment (Conaco et al. 2006; Packer et al. 2008). One target of miR-124a is the TF Sox9, which is crucial to glial lineage commitment (Cheng et al. 2009), while both miR-124a and miR-9 are capable of blocking generation of astrocytes by inhibiting STAT3 (signal transducer and activator of transcription 3) (Krichevsky et al. 2006). There are additional miRNAs, and likely more will be found, that are specific to neuronal or glial cell lineages (Juliandi et al. 2010). In fact, one study implicated the importance of a single miRNA (miR-219) in the switch from oligodendrocyte precursor cell (OPC) to fully myelinating oligodendrocyte (Dugas et al. 2010).

Liu et al. (2010) studied trans-differentiation of fibroblasts to myelinating oligodendrocytes by using ncRNAs in conjunction with true epigenetic reprogramming mechanisms. While they failed to produce myelinating oligodendrocytes, they did find that Dmmt and HDAC inhibitors (5'-azaC and TSA, respectively) were able to induce endogenous expression of oligodendrocyte-related genes without use of TFs (Liu et al. 2010; Sun et al. 2010). One potential roadblock to trans-differentiation was the unintended de-repression of transcriptional inhibitors of myelin genes by 5'-azaC and TSA. The authors suggested using miRNAs targeting the myelin inhibiting genes to improve trans-differentiation. Whether a combination of miRNAs, 5'-azaC, and TSA alone would be enough to help trans-differentiation into myelinating oligodendrocytes needs to be tested. Another possibility suggested involves adding SWI/SNF subunits or other chromatin remodelling complexes during reprogramming.

Similarly, and more effectively, Ambasudhan et al. (2011) used miRNA-124 in combination with two TFs (MYT1L and BRYN2) to generate functional neurons from adult human fibroblasts (Ambasudhan et al. 2011). This builds on the previously discussed finding that miRNA-124 aids in the development of neurons by hindering non-neuronal fate decisions. For trans-differentiation to become the primary method for generating all neural cell lineages, a better understanding of how epigenetic mechanisms regulate cell identity is required.

2.5 A Caveat

The size of mammalian genomes creates a practical difficulty in getting both exact and comprehensive data. Some researchers have found it easier, and more productive, to select regions of the genome where one expects to see important results. For histone modifications, being selective becomes especially important, given the vast number of possibilities. However, this will affect the quality of results, as well as ability to compare results. Further, when analyzing large scale and genome-wide datasets, important details can be lost, or unimportant details

magnified, depending on statistical methods. This was seen in an earlier study when statistical methods created conflicting results (Chin et al. 2009; Loh and Lim 2010). Clear and uniform statistical methods should be derived for epigenomic research to reduce potential conflicts. Finally, results by Meissner et al. (2008) indicate methylation level increases during cell culturing, causing potential aberrant hypermethylation. They argue that this may affect results from in vitro cell models. All of these should be considered when evaluating epigenetic maps.

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