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REVIEW

New insights into mechanisms that regulate DNA methylation patterning

Gabriella Ficz*

ABSTRACT

From a fertilised egg to a mature organism, cells divide and accumulate epigenetic information, which is faithfully passed on to daughter cells. DNA methylation consolidates the memory of the developmental history and, albeit very stable, it is not immutable and DNA methylation patterns can be deconstructed – a process that is essential to regain totipotency. Research into DNA methylation erasure gained momentum a few years ago with the discovery of 5-hydroxymethylcytosine, an oxidation product of 5-methylcytosine. The role of this new epigenetic modification in DNA demethylation and other potential epigenetic roles are discussed here. But what are the mechanisms that regulate deposition of epigenetic modifications? Until recently, limited direct evidence indicated that signalling molecules are able to modulate the function of epigenetic modifiers, which shape the epigenome in the nucleus of the cell. New reports in embryonic stem cell model systems disclosed a tight relationship between major signalling pathways and the DNA methylation machinery, which opens up exciting avenues in the relationship between external signals and epigenetic memory. Here, I discuss mechanisms and concepts in DNA methylation patterning, the implications in normal development and disease, and future directions.

KEY WORDS: 5-Hydroxymethylcytosine, DNA demethylation, Epigenetic memory, Reprogramming, Signalling pathways, Cancer stem cells

Introduction: cell identity, the epigenome and epigenetic barriers

Building up cell identity and epigenetic barriers in development

There are about 200 different cell types in the human body, each surrounded by the unique environment in which they are formed. Although these cells can have very diverse functions, they contain the same DNA sequence (with the exception of some immune cells) and originated from a single cell, the zygote. In the course of development, triggered by maternal determinants and extracellular signals, the process of differentiation renders cells with their own identity. Cell identity is defined by the sum of the molecular information a cell possesses and is surrounded by, information that perpetuates the phenotype at cell division. This identity is coupled to a unique epigenetic signature with the role to maintain cells 'locked in' to an identity and to only selectively respond to external signals. Therefore, even though we have one genome, we have hundreds of epigenomes. Waddington visualised in the 1950s how cell identity is formed in the development of multicellular organisms, whereby each cell (exemplified by a ball rolling down valleys separated by hills) acquires, processes and incorporates information from the nearby environment to exercise its genetic programme (see Hemberger et al., 2009; Ng et al., 2008). Cell plasticity is therefore a characteristic of early embryonic cells and a stepwise acquisition of information gradually restricts this potency and establishes epigenetic barriers to avoid transition from one state to another. Epigenetic barriers are molecular roadblocks that protect a cell from an uncontrolled transition into a less or more differentiated state by allowing a selective response to external signals. In essence, if a gene's promoter is permissive, with an open chromatin conformation, transcription factors will be able to bind unperturbed; in contrast, a gene promoter with a closed chromatin conformation will be refractive to the transcription factors. Combinations of open and closed chromatin at regulatory elements can constitute a roadblock or epigenetic barrier. In mammalian development, the first epigenetic barrier is established at the blastocyst stage where cells in the ICM [inner cell mass, from which embryonic stem (ES) cells are isolated *in vitro*] are separated functionally from the differentiated trophectoderm cells, which will contribute to the placenta (Ng et al., 2008). Expression of the gene *Elf5* is controlled by DNA methylation at its promoter region (the gene is shut down and hypermethylated in ES cells), but expression in the trophectoderm, where the promoter is unmethylated, precipitates a positive feedback loop to reinforce trophoblast identity. The ICM cells are pluripotent, which means that individual cells can be isolated and transplanted into a new blastocyst, where they will be able to colonise the embryo and contribute to any tissue in the organism including germ cells (Gardner, 1998). The same applies to ES cells cultured *in vitro*, as part of the test of pluripotency. But around implantation of the embryo, another epigenetic barrier is established when ICM cells start to differentiate. At this stage, a different type of stem cell can be isolated, called epiblast stem cells or EpiSCs (Tesar et al., 2007). However, when injected into blastocysts, EpiSCs will not contribute to the embryo to form chimeras, and are therefore distinct from pluripotent ES cells (Rossant et al., 1978). To reverse this process, EpiSCs need to be transfected with *Klf4* or other transcription factors to break the epigenetic barrier (Guo et al., 2009). Although fixed, cellular identity is not immutable (as discussed later), as exemplified by the mechanism of induced pluripotency. In this mechanism, terminally differentiated cells can be reprogrammed or dedifferentiated into pluripotent cells simply by overexpressing four pluripotency genes, genes that will rewire the entire transcription factor network (Takahashi et al., 2007). In this review, I focus on recent advances in understanding how methylation is deposited and the loopholes in epigenetic regulation that allow aberrant methylation to occur. An outstanding question is whether certain methylation patterns have the power to break down lineage barriers (explained below) or whether they are merely cementing and reinforcing these decisions.

Drivers of differentiation: are they genetic or epigenetic?

An interesting question arises in understanding the fundamental rules of establishing epigenetic patterns and barriers: is this

Centre for Haemato-Oncology, Barts Cancer Institute, London EC1M 6BQ, UK.

*Author for correspondence (g.ficz@qmul.ac.uk)

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programme driven and controlled by the genetic sequence or is the epigenome generated independently of the genetic sequence, within the same genomic element (e.g. promoters)? In other words, would promoters of Gene A and Gene B with very similar sequences acquire the same epigenetic information in development? An interesting study addressed this question and found that by inserting various 1000 bp sequences into the genome of mouse ES cells, these sequences recapitulated the DNA methylation patterns naturally found in ES cells and subtle genetic signatures attracted different DNA methylation patterns (Lienert et al., 2011). They also found that, by systematically reducing the length of the fragments inserted, below a certain length, the epigenetic signature could not be recapitulated. Therefore, this study revealed that the underlying genetic sequence is potentially a key driver for subsequent epigenetic events in normal development. Nevertheless, it does not rule out the possibility that temporal accumulation of methylation or other epigenetic modifications during development or caused by other signals (developmental or aberrant in disease) could change the trajectory for a DNA sequence and the gene it may control. As such, imprinting mechanisms exemplify an essential role that pre-determined epigenetic asymmetries (between identical sequences) play in normal development. Fertilisation involves fusion of the maternal and paternal genomes and although they have the same DNA sequence, the two pronuclei are different epigenetically as a result of genomic imprinting. The requirement for this asymmetry has been demonstrated by transplantation of a male or female pronucleus into haploid parthenogenetic eggs (McGrath and Solter, 1984; Surani et al., 1984). Essentially, eggs containing two female pronuclei or two male pronuclei did not complete normal embryogenesis, while the control transplantations (with one male and one female pronucleus) successfully developed to term. Therefore, epigenetic modifications play an equally important role in driving differentiation at different stages in development.

Is DNA methylation a leader or sealant?

DNA methylation is relatively fixed and is regarded as a bona fide epigenetic modification with a well-described mechanism (Goll and Bestor, 2005; Law and Jacobsen, 2010). Most of the methylation in somatic cells occurs in CG dinucleotides and, because of a high mutation rate of methylated cytosines through deamination and generation of TG, mammals have a reduced frequency of CGs compared with other dinucleotides. The distribution of CGs is unusual and while 70–85% of CGs in the genome are methylated (Ehrlich et al., 1982), CGs that are closely positioned within DNA sequences – also called CpG islands – are mostly unmethylated. It is notable that around 60% of mammalian promoters contain CpG islands (Antequera, 2003; Jones, 2012). This can represent a perfect system to effectively shut down genes through CGI hypermethylation, which is indeed the case for germline-specific genes. Although this mechanism is used in normal development, such as repression of pluripotency-associated genes when ES cells differentiate into neuronal progenitor cells (Mohn et al., 2008), hypermethylation of CGI promoters is observed at higher frequency in cancer and during ageing (Esteller, 2007; Issa, 2003), and opinions are divided as to whether this is a general mechanism in normal development (Bestor, 2000). It is emerging that in ES cells and in primordial germ cells, DNA methylation and gene expression are uncoupled (Ficz et al., 2013; Seisenberger et al., 2012). As such, global demethylation does not result in promiscuous transcription of genes, a phenomenon that could be characteristic of germ cells and pluripotent stem cells. In addition, many genes that become methylated at their promoter when ES cells differentiate have already been silenced in the ES cells (Mohn et al., 2008). Nevertheless, experimental induction of hypermethylation (discussed below) can silence genes, which indicates that DNA methylation can function both as leader and sealant. Quantitatively, it is unclear what the methylation threshold is beyond which the gene is inactivated. In previous work where reporter genes were targeted for methylation, 40% methylation was sufficient to inactivate a gene (Farthing et al., 2008; Li et al., 2007) although it is also possible that particular CpGs within the promoter bear more weight in gene expression control (Maeder et al., 2013).

Reversing the process: reprogramming in early development

Somatic cells are, to a large extent, in a terminally differentiated state constantly replenished through differentiation of adult stem cells. They acquire a stable identity and the local environment they are situated in reinforces this identity. Induction of reprogramming can alter this identity through developmental programmes and overexpression of transcription factors, which causes these cells to exit from their somatic programme into a less differentiated one. Such natural reprogramming has been observed *in vivo* and it is an intensely studied topic. There are two stages in development where global reprogramming happens: during germ cell differentiation and after fertilisation. The process is essential for resetting epigenetic imprints and re-establishing pluripotency (Lee et al., 2014). Reprogramming involves DNA methylation erasure in addition to major remodelling events of histone modification and gene expression. DNA demethylation can take place through two overlapping mechanisms: passive and active demethylation (see Fig. 1). During cell division, 5-methylcytosine (5mC) can be lost or erased when proteins that can copy it [DNA methyltransferases

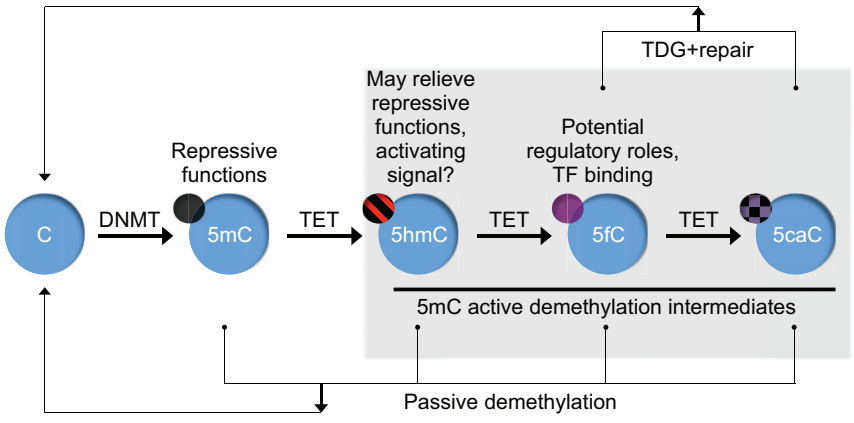

Fig. 1. Iterative 5mC oxidation and demethylation pathways. DNA methylation (5mC) is generated by DNA-methyltransferases (DNMTs), which can be oxidised by ten-eleven-translocation (TET) proteins to generate 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). All modifications can be lost through passive demethylation, which is replicative loss due to lack of maintenance during cell division. Active demethylation can also occur as thymine-DNA glycosylase (TDG) can excise 5fC and 5caC, which can be further repaired through the base excision repair pathway.

(DNMTs)] are absent or non-functional. This process is called passive demethylation. In contrast, active demethylation, where 5mC is lost and recycled to cytosine, can happen without DNA replication and cell division. In this latter case, 5mC is being iteratively oxidised by the TET (ten-eleven-translocation) proteins (Tahiliani et al., 2009), generating 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC; see Fig. 1) (Ito et al., 2011). 5fC and 5caC can be excised by thymine-DNA glycosylase (TDG) and repaired through the base excision repair pathway (He et al., 2011; Maiti and Drohat, 2011). Alternatively, active demethylation can also occur through deamination of 5mC to U catalysed by AID (AICDA) and APOBEC1 followed by base excision repair (Seisenberger et al., 2013). Active demethylation is particularly relevant in post-replicative cells, such as neurons, where high amounts of 5hmC have been detected (Kriaucionis and Heintz, 2009; Szwagierczak et al., 2010). It is becoming increasingly clear that these intermediates could have functional roles in controlling cell identity and the transition between these states (Hu et al., 2014; Song and He, 2013), particularly 5fC, which has been shown to attract and bind gene regulatory factors (Iurlaro et al., 2013; Spruijt et al., 2013).

Targeting epigenetic factors to DNA and chromatin

In vivo

More than a decade ago, DNA methyltransferases were recognised as being essential for mammalian development (Li et al., 1992; Okano et al., 1999), and this methylation, as a repressive modification, is needed for normal differentiation in both ES and adult stem cells. Several labs have since tried to understand the enzymatic activity of these proteins, how differential methylation is acquired and to what extent other proteins or transcription factors contribute to their biological function. DNMTs have been classified into two groups: maintenance and *de novo* enzymes. Maintenance methylases copy DNA methylation from the old strand template to the newly synthesised DNA strand during DNA replication, while *de novo* methylases do not need a template to methylate DNA. DNMT1 is considered to be the main maintenance methyltransferase, which propagates DNA methylation from mother cells to daughter cells (Goll and Bestor, 2005). DNMT1 shows an increased preference for a hemimethylated template in order to propagate existing methylation from the old strand to the newly synthesised strand (Yoder et al., 1997). DNMT3A and DNMT3B are *de novo* methyltransferases, responsible for methylation of DNA in the absence of a template. DNMT2 has been identified as having homology with the aforementioned DNMTs but is a tRNA methyltransferase and does not possess methyltransferase activity on DNA *in vitro*, and targeted deletion does not affect DNA methylation in ES cells (Okano et al., 1998). There is another member of the family, DNMT3L, which is best known for its role in imprint methylation maintenance during gametogenesis. In the absence of DNMT3L, genomic imprinting is lost (Bourc'his et al., 2001; Hata et al., 2002). DNMT3L interacts with DNMT3A and DNMT3B, facilitating their enzymatic activity (Chen et al., 2005; Chédin et al., 2002; Gowher et al., 2005; Suetake et al., 2004). The molecular mechanism and developmental role of DNA methylation have been the subject of intense research (Reik et al., 2001) but we are still lacking knowledge on how these enzymes are targeted to the DNA at different developmental stages to give rise to the cell type-specific patterns. The mechanisms reported so far include sequence-independent binding of DNMT3A and DNMT3B [due to protein domains such as the PWWP domain (Qiu et al., 2002)], interaction with transcription factors such as MYC (Brenner et al., 2005), and PML-RAR fusion (Di Croce et al., 2002). In addition, microRNA-facilitated recruitment has been reported in certain systems (Garzon et al., 2009). Finally, DNMTs were reported to interact with the tumour-suppressor retinoblastoma protein, RB (both in cancer cell lines and in normal calf brain); the sequence-specific transcriptional activator E2F1; the histone deacetylase HDAC1; the transcriptional repressor GCNF; the nuclear receptor COUP-TF1;the DNA-binding transcriptional repressor RP58; the methyl-CpG binding protein MeCP2; and the transcriptional co-repressor DMAP1 (Fuks et al., 2001; Gallais et al., 2007; Kimura and Shiota, 2003; Robertson et al., 2000; Rountree et al., 2000; Sato et al., 2006). Therefore, the picture is not so simple and despite the fact that we know how significant epigenetic depositions are in controlling cellular identity, in terms of their impact in development and disease, a unified mechanism for targeting of these enzymes is lacking.

The categorisation of the above-mentioned methyltransferases as either maintenance or *de novo* methyltransferases is an oversimplification as DNMT1 can have *de novo* activity as well (Bestor, 2000; Vertino et al., 1996). For example, methylation of the *Fas* gene upon induction of the *Ras* mutation does not involve DNMT3A and DNMT3B (Gazin et al., 2007). However, with DNMT1 being much more abundant and not having a significantly larger preference for hemimethylated substrates, it is highly likely that some *de novo* activity is carried out by DNMT1 (Yoder et al., 1997). For this reason, we cannot exclude the possibility that DNMT1 (or for that matter DNMT3 proteins) may play a role in *de novo* methylation deposition following instructions from signalling molecules (as described later).

Signalling pathways and control of epigenetic modifiers

In broad terms, both signalling and epigenetic mechanisms act in order to control gene expression in cells. Nevertheless, there is a gap in our knowledge of how these two mechanisms link and overlap (see below). There have been reports that showed evidence of such an intersection, for example the Wnt/APC/β catenin/TCF pathway controlling DNMT1 protein levels (Campbell and Szyf, 2003). In recent years, this phenomenon has become more evident as studies found that post-translational modifications generated by AKT1 and PKC can influence DNMT1 activity, its affinity for DNA and its interaction with other proteins (Hervouet et al., 2010; Lavoie et al., 2011). They showed that DNMT1 is phosphorylated at Ser127 and Ser143, which impairs the protein's DNA methylation enzymatic activity and its interaction with PCNA/UHRF1. In addition, DNMT1 protein levels can be negatively modulated by SET7-mediated methylation and positively modulated by LSD1-mediated demethylation (Estève et al., 2009; Wang et al., 2009). These studies provided a mechanism whereby signalling events can lead to aberrant DNA methylation that precede genome instabilities and could facilitate mutational events in adult stem and progenitor cells.

More recently, in ES cells, such a relationship has been uncovered and has shown a strong effect on global methylation levels (Ficz et al., 2013; Habibi et al., 2013; Takashima et al., 2014). In mouse ES cells, exit from pluripotency is controlled by FGF4 signalling, and inhibition of this pathway with small molecule inhibitors to ERK1/2 and GSK3β (called 2i inhibitors) induces the so-called ground state pluripotency (see Fig. 2), a state resembling cells in the ICM (Ying et al., 2008). ES cells can therefore be captured in at least two different states: the primed state (where cells are on the verge of exiting from pluripotency and are a heterogeneous population) and the ground state (a more homogeneous population, which might mimic more the *in vivo* condition in the ICM); these two states are interconvertible (Fig. 2). 2i inhibition leads to a widespread loss of

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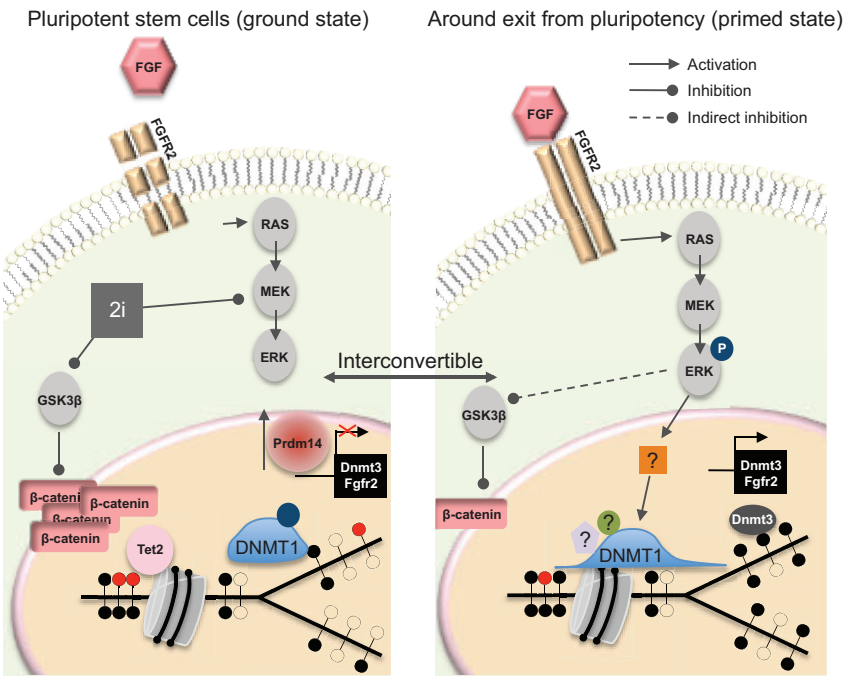

Fig. 2. Intersection of the FGF–ERK signalling pathway with the DNA methylation machinery in embryonic stem cells. Inhibition of the ERK1/2 and GSK3β signalling using 2i inhibitors in mouse embryonic stem cells induces transition into the ground state, which is associated with overexpression of PRDM14, widespread global demethylation and increased oxidation. Removal of 2i primes cells to exit from pluripotency but these two states as well as demethylation and remethylation are interconvertible. DNMT1 is more tightly associated with DNA and chromatin in the primed state. Question marks indicate unknown mechanisms, proteins and post-translational modifications.

genomic methylation and, as mentioned above, this does not result in promiscuous overexpression of demethylated genes; therefore, DNA methylation and gene expression are uncoupled in this system. Signalling inhibition was found to acutely downregulate the *de novo* methylation machinery while DNMT1 levels remained unchanged, although full function of DNMT1 was not verified. Nevertheless, increased oxidation by TETs upon 2i inhibition facilitated passive loss of methylation, which resulted in genome demethylation (Ficz et al., 2013). Recently, a similar effect was reported in human ES cells, indicating a generality for this phenomenon in mammals (Takashima et al., 2014). In this case, in addition to the 2i inhibitors, a pan-PKC inhibitor was added to stabilise the human ground state pluripotency transcription factor circuitry and this was also associated with widespread global demethylation (Takashima et al., 2014). The mechanism that controls demethylation in the ground state pluripotency is currently under investigation. PRDM14 is involved in the early stages of germ cell reprogramming and the ES cell system shows that it plays a role in reprogramming to the ground state pluripotency as well (Fig. 2). Downstream factors from ERK1 and ERK2 will probably be involved in modulating DNA methylation patterning.

Experimental epigenetic editing

One way of addressing the question of whether DNA methylation is a driver or a sealant is to exogenously target the modification to regions of interest and analyse the outcome. This is absolutely key in mechanistically understanding the functional relevance of the DNA methylation dynamics as far too often changes in DNA methylation are described correlatively. The term epigenetic editing was coined by the Rotts lab and involves various techniques whereby mitotically stable epigenetic changes are targeted to specific sequences in the genome using effector molecule fusions to DNA binding molecules (de Groote et al., 2012). The process involves DNMT proteins being directed to DNA sequences with systems such as UAS-GAL4, zinc fingers (ZNFs) or fusion with the DNA binding domains of proteins. Fusion of DNMT proteins with inactive Cas9 is also theoretically possible, where Cas9 is the RNA-directed endonuclease, as part of the recently developed CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats) system. This last method, which is technically more versatile and easy to use for studying epigenetic modifications and their direct consequence on cell physiology, opens up a whole new world of possibilities.

There are advantages and disadvantages in each system; gene-specific targeting of methylation has been validated using methods such as GAL4-DNMT fusion proteins (Farthing et al., 2008; Li et al., 2007). The drawback of this methodology is that the target genome needs to be modified by inclusion of the UAS repeats, which is necessary in order to attract GAL4 fusion proteins. ZFN and transcription activator-like effector (TALE) targeting systems circumvent this problem so that virtually any genomic DNA sequence can be targeted by design (Bibikova et al., 2003; Moscou and Bogdanove, 2009). Although flexible, these methods are rather laborious and will probably be outcompeted in terms of cost and design by CRISPR/Cas9 targeting systems (Cong et al., 2013). CRISPR/Cas9 facilitates RNA-guided site-specific targeting of DNA cleavage by Cas9 nucleases. Essentially, catalytically inactive *Cas9* fused to *Dnmt* or *Tet* catalytic domain sequences can be directed to any genomic location, using a guide-RNA (designed separately for each genomic locus). Thus, in the future, these will be useful tools available to interrogate specific methylations for functional relevance in a particular experimental system.

Differentiation and reprogramming: opportunities for change

Opportunities for phenotypic variation

Ontogenesis is a fast forward picture of evolution but early embryogenesis is also an opportunity to integrate environmental, *in utero* signals in a continuously changing environment. Recent understanding of early embryo differentiation suggests that epigenetic mechanisms play a role in acquiring environmental information, and create diversity and opportunities during cell fate decision making, leading to increased adaptation of cells to their environment (Lee et al., 2014). This is potentiated by the oxidative capacity of the TET proteins as described below. Essentially, DNA methylation is erased after fertilisation and progressively accumulates as cells differentiate. This accumulation can be reversed

or manipulated by the three isoforms of TET proteins (TET1, TET2 and TET3). TET1 and TET2 are highly expressed in ES cells but decline when cells differentiate and exit from the pluripotency niche, while TET3 has the opposite pattern. The phenotypes of *Tet* knockout mice indicate an important and interesting role for the oxidation of 5-methylcytosine in early development and phenotypic variation. As such, mice lacking *Tet1* and *Tet2* genes survive but have considerable imprinting defects and many of them die perinatally (Dawlaty et al., 2013). Mice lacking *Tet2* develop blood cancers in adulthood (Ko et al., 2011; Li et al., 2011; Moran-Crusio et al., 2011; Quivoron et al., 2011). Homozygous deletion of *Tet3* is neonatal lethal while conditional deletion of maternal *Tet3* impairs paternal genome reprogramming and causes developmental failure of the embryo (Gu et al., 2011). Most interestingly, absence of all three *Tet* genes is incompatible with normal development (Dawlaty et al., 2014). When ES cells are injected in the blastocysts, the wild-type cells contribute and integrate in all tissues while *Tet* knockout ES cells, although viable and present in the implanted embryo, fail to compete with normal ES cells and eventually die during normal development. This indicates that around implantation, multicellular organisms allow cells to compete with each other, based on plasticity, in response to environmental signals. Indeed, the recent theoretical concept is that mammalian genomes have evolved to integrate and propagate genes that overall maintain the mean phenotype but increase the propensity to phenotypic variability, fitness and disease (Feinberg and Irizarry, 2010).

Opportunities for disease: cancer initiation

In an ever-changing environment, multicellular organisms have developed mechanisms to conserve structural integrity while maintaining sufficient flexibility to react and adapt to new environments when necessary. One such example is adult stem cells in mammals, cells that reconstruct damaged tissues and replace terminally differentiated cells. They are multipotent cells, able to differentiate into several cell types depending on the signals received within their local environment. By definition, adult stem cells have a more restricted potential compared with the pluripotent ES cells; therefore, they will be refractory to lineage-specific signals other than those specific for their tissue (Morrison and Spradling, 2008). Each adult stem cell first differentiates into an early progenitor and subsequently more mature progenitors, which will further diversify. Along this process of integrating external signals during adult stem cell differentiation, there are opportunities to receive abnormal instructions, which could result in derailed differentiation of these cells and disruption of barriers separating lineages. The discovery by Yamanaka and colleagues that differentiated cells can be reprogrammed into an induced pluripotent cell by overexpressing a defined set of transcriptional factors not only had consequences in stem cell and regenerative medicine but also demonstrated a fundamental property of cells in general: deploying gene regulatory mechanisms in an inappropriate developmental context can have devastating consequences. An example is the emergence of cancer stem cells (CSCs), cells that give rise to tumours. The concept of CSCs is controversial, certainly when they are proposed to derive solely from adult stem cells (Jordan, 2009). Irrespective of their origin, CSCs are capable of self-renewal and differentiation, similar to other stem cells, and generate phenotypically heterogeneous tumours (Reya et al., 2001). Moreover, it is not always the case that mutations are elemental in the induction of tumorigenesis and CSCs can arise from differentiated cells too as was recently demonstrated in a very exciting study (Ohnishi et al., 2014). In this mouse model, where expression of the four Yamanaka reprogramming factors (*Oct3/4*, *Sox2*, *Klf4* and *c-Myc*) can be induced with doxycycline, long-term induction of these factors led to the generation of multiple tumours, some of them resembling Wilms tumour in the kidney (Ohnishi et al., 2014). Although these processes are probably rare *in vivo*, they nevertheless highlight the potential that such disturbances may happen and can potentially increase with age when cancers are more prevalent. No cancer-related genetic mutations were detected in these tumours; therefore, tumorigenesis could have resulted from non-genetic or epigenetic events.

Concluding remarks

These insights have highlighted the need to explore in more detail the relationship between signalling pathways and epigenetic modulators in order to understand how epigenetic memory is controlled and inherited during cell division, potentially leading to cell identity alterations. While epigenetic memory is the sum of the developmental history, it appears that there are various routes to destabilise the barriers, rewire the epigenome and initiate alternative cell fates. These questions are highly relevant in ageing and cancer, where such barriers might be under increased environmental pressure compared with normal tissues. Recent data indicate that genetic mutations are not compulsory for cancer initiation and it is highly likely that epigenetic aberrations are at the root of subsequent genomic instabilities that enable genetic mutations to occur. This is emphasised by recent data in haematological malignancies, whereby serial sequencing during tumour evolution found that cancer-related mutations in tumour suppressors occur subsequent to mutations in epigenetic modulators. Therefore, it is possible that aberrations in epigenetic patterning are the culprit for initiation of tumourigenesis in the absence of genetic mutation or may allow mutations to happen in the context of, for example, genomic hypomethylation. DNA demethylation and reprogramming are necessary to break the epigenetic barrier in order to transit between different cell identity states but the presence of initiating signals is necessary in order to ignite a programme and establish transcription factor circuitry. Therefore, finding the drivers and subsequent mediators that control and modulate the targeting of epigenetic factors should be an attractive focus of future research to understand the complex patterning of the epigenome and its influence on phenotypic variation.

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Competing interests

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