Regenerating the epigenome

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The ability of some organisms to regenerate parts of their body has fascinated scientists for decades. The process of regeneration depends on the potential of certain cells to proliferate and contribute to the formation of new tissue. Organisms have evolved two strategies by which to achieve this: the maintenance of adult stem cells and the induction of stem-cell properties in differentiated cells. In both cases, cells must undergo extensive epigenetic reprogramming to attain the specialized functions of the new tissue. Ultimately, the regenerative capacity of a tissue might depend on the plasticity of the cellular epigenome, which determines the ability of the cell to respond to injury-related signals. Understanding this epigenetic plasticity will allow the development of strategies to stimulate the regeneration of damaged tissues and organs in humans.

Keywords: epigenetics; chromatin; regeneration; stem cell; reprogramming

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See Glossary for abbreviations used in this article.

Chromatin modifications regulate gene expression

The nucleosome is the fundamental unit of chromatin. It is composed of two copies each of four core histones—H2A, H2B, H3 and H4—which are wrapped by 146 bp of DNA. The amino-terminal tails of histones are relatively accessible to enzymatic modifications such as acetylation, methylation, phosphorylation, ubiquitination and SUMOylation. Acetylated histones can induce the structural relaxation of chromatin and act as binding sites for proteins that participate in transcriptional activation. Other modifications, such as methylation, act as docking sites that promote the recruitment and stabilization of effector protein complexes, instead of altering the chromatin structure *per se* (reviewed in Kouzarides, 2007). In a similar manner, DNA methylation—which takes place at cytosine residues within CpG dinucleotides at gene promoters—usually correlates with transcriptional repression and mediates its effect by blocking the binding of transcription factors and/or facilitating the assembly of repressor complexes that contain methylated DNA-binding domains (reviewed in Shoemaker *et al*, 2010).

The ultimate effect of modified histones or DNA is usually the recruitment or stabilization of activating and repressing effector

complexes. These can mediate: the acetylation or deacetylation of histones; nucleosome mobility or even eviction to facilitate the compaction or relaxation of chromatin; or recruitment of linker histones and structural non-histone proteins that participate in chromatin compaction and assembly of higher order structures. The relaxation of chromatin makes DNA more accessible for the binding of transcription factors and facilitates the recruitment and processivity of the RNA polymerase (Hodges *et al*, 2009).

Different regions of the genome are organized into chromatin structures that are broadly classified as euchromatin or heterochromatin. Euchromatin contains actively transcribed genes that are enriched in histone H3 trimethylated at Lys4 (H3K4me3). Heterochromatin, which is enriched in hypoacetylated histones and methylated DNA, is classified into two subgroups: constitutive and facultative. Constitutive heterochromatin contains highly condensed regions of the genome that are enriched in H3K9 methylation and have few genes, such as centromeric regions. Facultative heterochromatin refers to regions of the genome that are rich in genes that are silenced, but can be activated in certain situations or cell types, and are therefore susceptible to regulation. Overall, these areas are marked with H3K27me3 and include many developmental regulator genes bound by the Polycomb group (PcG) of proteins.

The epigenetic landscape of embryonic stem cells

Embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of the preimplantation embryo and are characterized by their ability to self-renew and give rise to almost any cell type of the adult organism, a property known as pluripotency.

ESCs have characteristic chromatin modification patterns called bivalent domains at the regulatory regions of developmental genes. These are characterized by large regions of H3K27me3 that coexist with smaller regions of H3K4me3 around the transcriptional start site (Fig 1). The coexistence of these antagonistic marks has been suggested to silence developmental genes in ESCs, while keeping them poised for activation upon the initiation of specific developmental pathways (Azuara *et al*, 2006; Bernstein *et al*, 2006). The PcG complex controls these domains by mediating the H3K27 methylation mark (Azuara *et al*, 2006; Boyer *et al*, 2006; Lee *et al*, 2006) and the ubiquitination of histone H2A, leading to a poised RNA polymerase II configuration (Stock *et al*, 2007).

The physiological function of bivalent domains might be to maintain the accessibility of important regulatory sequences to the binding of relevant transcription factors at early stages of differentiation. These regulatory areas subsequently close up and become

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Fig 1 | Model for resolution of bivalent domains during regeneration. Regeneration signals, such as inflammation, can induce changes in the expression levels of subunits of the PcG complexes (PRC1 and PRC2) and the H3K27 demethylase Jmjd3. These changes help to tip the balance towards the resolution of bivalent domains. This mechanism seems to be common during the differentiation of embryonic and adult stem cells and fin regeneration in zebrafish. Active marks are shown in blue and repressive marks in red. Bmi1, B-cell-specific Moloney murine leukaemia virus integrations site 1; Eed, embryonic ectoderm development; Ezh2, enhancer of zeste homologue 2; Jmjd3, Jumonji domain containing 3; Mll, myeloid/lymphoid or mixed-lineage leukaemia; Pol II, RNA polymerase II; PRC1/2, polycomb repressive complex 1/2; Ring1, ring finger protein 1; Ser2-P and Ser5-P, phosphorylated serine residues 2 and 5 in the carboxy-terminal domain of Pol II; Suz12, suppressor of zeste 12 homologue.

heterochromatinized (Gargiulo *et al*, 2009). In these regions, the bivalent domains resolve into H3K27 methylation (Cui *et al*, 2009; Mikkelsen *et al*, 2007) and further repression is often ensured by the acquisition of other repressive marks, such as H3K9 or DNA methylation (Hawkins *et al*, 2010). Therefore, these regions become condensed and refractory to stimulation, contributing to the definition and preservation of cell identity. Importantly, a significant number of bivalent domains can remain unresolved after differentiation, determining the potency of the adult cells (Cui *et al*, 2009; Mikkelsen *et al*, 2007; Mohn *et al*, 2008).

Epigenetic regulation of adult stem cells

Adult stem cells can participate in the natural homeostasis of adult tissues—in which they mediate the continuous replacement of differentiated cells—or be quiescent and respond to injury signals when needed to regenerate tissue (Li & Clevers, 2010). Somatic stem cells, which can self-renew and differentiate into a limited number of cell types (Morrison & Kimble, 2006), have been identified in most tissues, including blood, brain, muscle, skin and gut.

Several lines of evidence suggest that, similarly to ESCs, adult stem cells can maintain their differentiation potential due to the presence of genes marked with bivalent domains that are ready to be activated during differentiation. First, although neuronal progenitor cells (NPCs) have undergone extensive DNA methylation in comparison to ESCs, they still retain bivalent domains in several genes (Mohn *et al*, 2008). Comparisons between ESCs, NPCs and mouse embryonic fibroblasts (MEFs) indicate that genes that are bivalent in ESCs and are involved in gliogenesis and neurogenesis, either remain bivalent or resolve to H3K4 methylation in NPCs, whereas

they resolve to H3K27me3 alone in MEFs (Mikkelsen *et al*, 2007). This suggests a link between the presence of bivalent marks and cell potency. Second, the resolution of bivalent domains during the differentiation of haematopoietic stem cells (HSCs) to erythrocyte precursors correlates with changes in the expression of crucial transcription factors and surface markers that contribute to the terminal differentiation of these cells (Cui *et al*, 2009; Fig 2). Therefore, ESCs contain several bivalent genes that are representative of all three embryonic germ layers, whereas adult stem cells have fewer of these genes, which might explain their limited potential.

Some PcG proteins are highly expressed in self-renewing adult stem cells. Ezh2, for example, is strongly expressed in skin stem cells and NSCs, and downregulated during their differentiation (Ezhkova *et al*, 2009; Sher *et al*, 2008). Bmi1, a component of the PRC1 complex, is also highly expressed in intestinal cells and HSCs (Park *et al*, 2003; Sangiorgi & Capecchi, 2008). Loss-of-function experiments suggest that PcG proteins have crucial roles in the maintenance of several types of adult stem cell through two pathways (Table 1). First, PcG proteins promote cell proliferation and stem-cell self-renewal by repressing the p16Ink4a/p19Arf locus and thereby counteracting cell senescence (Ezhkova *et al*, 2009; Molofsky *et al*, 2003; Park *et al*, 2003). Second, Polycomb-repressive complexes contribute to the maintenance of differentiation genes that are repressed and poised for activation during differentiation (Oguro *et al*, 2010; Pereira *et al*, 2010).

An injury can cause inflammation in the stem-cell niche, thereby releasing signals—such as cytokines—that induce regeneration. The way in which injury cues are converted into epigenetic information is unknown, but changes in the expression of crucial chromatin

Fig 2 | Bivalent domains have a role in defining the potency of the haematopoietic lineage. This example shows that the orchestrated resolution of bivalent domains contributes to the determination of cell identity and plasticity. The preservation of bivalent domains at certain genes determines the level of cellular potency. ESC, embryonic stem cell; HSC, haematopoietic stem cell. See glossary for gene name abbreviations.

remodellers in response to these signals seem to have a role. The downregulation of PcG protein expression can contribute to the differentiation of adult stem cells by releasing the repression of differentiation genes (Ezhkova *et al*, 2009). Further, inflammatory cytokines have been described to induce the expression of the H3K27 demethylase Jmjd3 (De Santa *et al*, 2007). Similarly, the coordinated downregulation of several PcG proteins and upregulation of the H3K27 demethylases Jmjd3 and Utx contribute to the activation of wound-induced genes during wound healing (Shaw & Martin, 2009).

Overall, the balance between histone lysine methyltransferases and demethylases seems to be crucial for the maintenance and resolution of bivalent domains (Fig 1). For example, NSCs deficient for the H3K4 methyltransferase Mll1 can efficiently self-renew and differentiate into glia but not into neurons, due to defects in the removal of H3K27 methylation marks from poised neuronal genes. This supports a model in which Mll1 facilitates the recruitment of Jmjd3 to these genes during neuronal differentiation (Lim *et al*, 2009; Fig 1). Interestingly, regeneration signals can directly modulate the activity of chromatin remodellers. Injury signals have recently been shown to induce the phosphorylation of Ezh2 by TNF-activated p38 α kinase. This promotes the displacement of the PcG complex from poised differentiation genes to the muscle satellite-cell marker Pax7, thereby contributing to the coordinated activation of bivalent genes and silencing of potency factors (Palacios *et al*, 2010). The regeneration of *Drosophila* imaginal discs after injury similarly leads to the activation of the JNK signalling pathway and, thereby, to PcG inhibition. PcG proteins maintain cellular identities in the fly imaginal discs, and reduced PcG expression enhances the frequency of leg-to-wing transdetermination events during disc regeneration (Klebes *et al*, 2005; Lee *et al*, 2005).

Overall, adult stem cells maintain the presence of bivalent domains at crucial differentiation genes, through the balanced activity of histone lysine methyltransferases and demethylases at these domains. Injury signals can modulate these activities and tip the balance towards the resolution of bivalent domains, thereby inducing differentiation (Fig 1).

Regeneration by dedifferentiation and transdifferentiation

Some tissues are able to regenerate independently of stem or progenitor cells. This involves a process of dedifferentiation or transdifferentiation—sometimes through an undifferentiated intermediate—of specialized cell types (Fig 3).

Zebrafish hearts can regenerate when up to 20% of the ventricle is amputated. During regeneration, the newly formed heart-muscle cells are derived from the dedifferentiation and proliferation of pre-existing cardiomyocytes (Jopling *et al*, 2010; Kikuchi *et al*, 2010). After heart injury, the expression of several cell-cycle regulators is activated in cardiomyocytes, promoting proliferation (Jopling *et al*, 2010; Lien *et al*, 2006; Sleep *et al*, 2010). Dedifferentiation is characterized by the reactivation of the embryonic cardiogenesis gene *Gata4* and decreased expression of sarcomeric components, which leads to the disassembly of the sarcomeric structure (Jopling *et al*, 2010; Kikuchi *et al*, 2010; Sleep *et al*, 2010; Fig 3).

During limb regeneration in urodeles, a progenitor cell zone known as blastema is formed and regenerates the missing portion of the limb. This group of cells gives rise to epidermis, dermis, muscle, nerves, blood vessels and skeleton. As the blastema seems to be a homogeneous group of undifferentiated cells, it has been speculated that these cells are pluripotent. However, the cells in the blastema have recently been shown to be derived from several differentiated

Table 1 | Chromatin factors involved in stem-cell homeostasis and regeneration

Complex	Factor	Cell type	Loss-of-function phenotype
PRC1	Ring1	ESC	Premature differentiation (Stock <i>et al</i> , 2007)
		NSC	Impaired self-renewal and premature differentiation (Roman-Trufero <i>et al</i> , 2009)
	Bmi1	HSC	Impaired self-renewal and derepression of differentiation genes (Oguro <i>et al</i> , 2010; Park <i>et al</i> , 2003; van der Lugt <i>et al</i> , 1994)
		NSC	Impaired self-renewal (Molofsky <i>et al</i> , 2003; Zencak <i>et al</i> , 2005)
		InSC	Impaired self-renewal (Sangiorgi & Capecchi, 2008)
PRC2	Ezh2	ESC	Derepression of differentiation genes and impaired differentiation (Shen <i>et al</i> , 2008)
		NSC	Derepression of differentiation genes (Pereira <i>et al</i> , 2010)
		HepSC	Premature differentiation (Aoki <i>et al</i> , 2010)
	Eed	ESC	Derepression differentiation genes and impaired differentiation (Azuara <i>et al</i> , 2006; Boyer <i>et al</i> , 2006; Shen <i>et al</i> , 2008)
	Suz12	ESC	Derepression of differentiation genes and impaired differentiation (Lee <i>et al</i> , 2006; Pasini <i>et al</i> , 2007)
		NSC	Premature differentiation (Pereira <i>et al</i> , 2010)
BAF	Brg1	ESC	Impaired self-renewal and differentiation (Kidder <i>et al</i> , 2009; Schaniel <i>et al</i> , 2009)
		NSC	Precocious neuronal differentiation (Matsumoto <i>et al</i> , 2006)
NuRD	Mi-2b	HSC	Self-renewal and differentiation deregulation (Yoshida <i>et al</i> , 2008)
Mll	Jmjd3	NSC	Impaired differentiation (Burgold <i>et al</i> , 2008; Jepsen <i>et al</i> , 2007)
		fin	Impaired fin regeneration (Stewart <i>et al</i> , 2009)
	Mll1	NSC	Impaired neuronal differentiation (Lim <i>et al</i> , 2009)

BAF, BRG1-associated factors; ESC, embryonic stem cell; InSC, intestinal stem cell; HepSC, hepatic stem cell; HSC, haematopoietic stem cell; Mll, myeloid/lymphoid or mixed-lineage leukaemia; NSC, neural stem cell; NuRD, nucleosome remodelling and histone deacetylation repressor complex; PRC1/2, polycomb repressive complex 1/2. See the Glossary for remaining gene name abbreviations.

cell types and retain a memory of their origin, as they redifferentiate into the same type of cell in the regenerating limb. Dedifferentiated cells derived from cartilage, for example, only give rise to cartilage cells and do not transdifferentiate into other cell types. Therefore, the blastema seems to be formed by a heterogeneous population of undifferentiated cells with restricted potential (Kragl *et al*, 2009).

The limited dedifferentiation that cells undergo during regeneration seems to be frequently characterized by the silencing of tissue-specific genes (Jopling *et al*, 2010; Sleep *et al*, 2010). In the zebrafish caudal fin, amputation also leads to the reactivation of developmental regulators, such as *Shh* and the *Msx* family of genes (Akimenko *et al*, 1995; Laforest *et al*, 1998). A recent report suggests that many of these genes are marked with bivalent domains in the zebrafish fin and, during regeneration, the H3K27me3 mark is removed by members of the Jmjd3 family (Stewart *et al*, 2009; Fig 1). Accordingly, the regulatory DNA regions of the *Shh* gene have been shown to be hypermethylated in adult frogs, which cannot regenerate their limbs, but hypomethylated in tadpoles, which can do this (Yakushiji *et al*, 2007). Therefore, embryonic genes involved in regeneration might remain poised for activation in regenerating animals and irreversibly silenced in non-regenerating animals.

Only a few cases of natural transdifferentiation have been described. Glucagon-producing α -cells, for example, can transdifferentiate into pancreatic insulin-producing β -cells after ablation of β -cells with diphtheria toxin (Fig 3). This process does not seem to involve the existence of an undifferentiated intermediate, as cells that co-express glucagon and insulin are frequently observed (Thorel *et al*, 2010). However, transdifferentiation can also occur through an undifferentiated intermediate, as is the case during the regeneration

of the eye lens in newts (Tsonis *et al*, 2004). Lens regeneration takes place through transdifferentiation of the pigmented epithelial cells (PECs) of the iris. After removal of the lens, the PECs re-enter the cell cycle, dedifferentiate, and lose their characteristic pigmentation. During this early stage, the expression of developmental regulators such as Pax6 and Sox2 is activated. Later, the dedifferentiated proliferating PECs start to express crystallins and differentiate into lens fibres (Fig 3).

Taken together, these results suggest that the process of dedifferentiation during regeneration involves the silencing of tissue-specific genes, as well as the induction of genes involved in embryonic programmes and control of the cell cycle. These changes in gene expression might facilitate the acquisition of plasticity that allows cells to proliferate and rearrange into the new structures. In most cases, the cells do not seem to gain new potencies after dedifferentiation. Importantly, the few reported cases of natural transdifferentiation involve a switch in identity between closely related cell types. This suggests that a more permissive epigenetic context favours the regeneration of certain organs through dedifferentiation or transdifferentiation, whereas important epigenetic restrictions preclude extreme changes of cell identity.

Engineered transdifferentiation and dedifferentiation

Understanding the basic mechanisms of self-renewal, pluripotency and regeneration has allowed the manipulation of cell identity, which is achieved through the overexpression of crucial transcription factors.

The discovery of master transcription factors that are able to activate whole programmes of tissue-specific gene expression has

A Heart regeneration

B Lens regeneration

C Pancreas regeneration

Fig 3 | Dedifferentiation and transdifferentiation during *in vivo* regeneration. (A) Zebrafish heart regeneration involves limited dedifferentiation and proliferation of pre-existing cardiomyocytes. (B) Lens regeneration in newts proceeds by transdifferentiation of iris cells to crystalline cells, a process that involves a dedifferentiation and proliferation step. (C) The regeneration of pancreatic β -cells from pre-existing α -cells after massive β -cell loss in mice takes place through direct transdifferentiation, is independent of proliferation, and includes an intermediate stage in which cells co-express both the α -cell and β -cell programmes. Cmlc2, cardiac myosin light chain 2; Gata4, GATA binding protein 4; Pax6, paired box 6; Plk1, polo-like kinase 1; Prox1, prospero homeobox 1.

allowed strategies to change cellular identity by forcing the expression of these factors. For example, MYOD is able to induce the phenotypic conversion of fibroblasts to myogenic cells (Davis *et al*, 1987). Similarly, the overexpression of PPAR γ 2—an adipocyte-specific nuclear hormone receptor—can induce adipogenesis in fibroblasts (Tontonoz *et al*, 1994). In addition, the expression of only three factors—Ascl1, Brn2 and Myt1l—can convert fibroblasts into functional neurons *in vitro* (Vierbuchen *et al*, 2010), and the

transcription factors Gata4, Mef2c and Tbx5 in combination can reprogramme fibroblasts to become cardiomyocyte-like cells (Ieda *et al*, 2010).

Transdifferentiating one cell type into another *in vivo* is a challenge for regenerative medicine. Several studies report that it is possible to generate cells that produce insulin from non-insulin-producing cells in mice. The transduction of mouse liver with an activated form of *Pdx1*—a central regulator of pancreatic development—causes the

Sidebar A | In need of answers

- (i) How do signalling events mediate the activation or repression of specific genes during regeneration?
- (ii) Which genes are crucial for the regeneration process?
- (iii) Which epigenetic signatures predispose the crucial genes involved in regeneration to regulation?
- (iv) Can we identify crucial epigenetic barriers that impair regeneration in humans, and develop strategies to overcome them?
- (v) Could the use of chemicals that target the epigenetic machinery be used to boost regeneration in non-regenerating animals?

expression of many pancreatic markers, including insulin, in liver cells (Ferber *et al*, 2000). *Pdx1*, together with the transcription factors Ngn3 and Mafa, can also reprogramme differentiated pancreatic exocrine cells into cells similar to adult mouse β -cells (Zhou *et al*, 2008).

In vitro reprogramming to undifferentiated, multipotent states generates cells that can differentiate into diverse cell types. The reprogramming of somatic cells to pluripotency is a dramatic example of such manipulations. A decade ago, nuclear transfer experiments were the first to show that the differentiated phenotype of adult cells can be fully reversed (Wilmot *et al*, 1997). More recently, induced pluripotent stem cells (iPSCs) were derived from somatic cells by overexpressing a combination of four transcription factors: Oct4, Sox2, Klf4 and c-Myc (Takahashi & Yamanaka, 2006). This seminal study led the way for the use of other, more simplified transcription-factor combinations (Nakagawa *et al*, 2008; Wernig *et al*, 2008).

The low efficiency of somatic-cell reprogramming using transcription factors can be explained, at least in part, by the existence of epigenetic barriers that preserve the identity of adult cells. First, the presence of an inaccessible repressive chromatin environment at the regulatory regions of the endogenous pluripotency genes impairs their transcriptional activation by the exogenous factors (Sridharan *et al*, 2009). Second, after the reactivation of the endogenous pluripotency network, the epigenetic memory of the cell-of-origin needs to be erased. This is achieved by the re-establishment of bivalent domains at developmental genes, which implies that the tissue-specific genes that were expressed in the cell-of-origin are silenced and, perhaps more importantly, a permissive chromatin structure is regained at those differentiation genes that were irreversibly repressed. The importance of the proper reprogramming of bivalent domains is illustrated by a study describing that iPSCs derived from non-haematopoietic cells have reduced potential to generate blood, owing to remnant DNA methylation at loci required for haematopoietic lineage specification (Kim *et al*, 2010). Furthermore, the aberrant expression of bivalent genes in mouse iPSCs is inversely correlated with their ability to give rise to viable animals by tetraploid complementation (Boue *et al*, 2010).

Regeneration and reprogramming compared

The fact that cells can revert their differentiated status—both during regeneration and during reprogramming to pluripotency—has prompted researchers to compare these processes and, more specifically, to evaluate the potential involvement of reprogramming factors in regeneration. The transcription of Sox2, Klf4 and c-Myc has been reported to be upregulated during lens and limb regeneration in newts (Maki *et al*, 2009). In addition, Oct4 and Sox2 have been

Glossary

Ascl1	achaete-scute complex homologue 1
Bmi1	B-cell-specific Moloney murine leukaemia virus integrations site 1
Brg1	LysR family transcriptional regulator
Brn2	POU class 3 homeobox 2
c-Myc	v-Myc myelocytomatosis viral oncogene homologue
C/EBP	emopamil binding protein (sterol isomerase)
Eed	embryonic ectoderm development
Ezh2	enhancer of zeste homologue 2
Gata4	GATA binding protein 4
Jmjd3	Jumonji domain containing 3
JNK	jun amino-terminal kinase
Klf4	kruppel-like factor 4
Mafa	v-Maf musculoaponeurotic fibrosarcoma oncogene homologue A
Mef2c	myocyte enhancer factor 2c
Meis1	meis homeobox 1
Mi-2b	chromodomain helicase DNA binding protein 4
Mll1	myeloid/lymphoid or mixed-lineage leukaemia 1
Msx	homeobox protein Msx
Myt1l	myelin transcription factor 1-like
Nanog	nanog homeobox
Ngn3	neurogenin 3
Oct4	POU class 5 homeobox 1
Pax6/7	paired Box 6/7
Pdx1	pancreatic and duodenal homeobox 1
PPAR γ 2	peroxisome proliferator-activated receptor γ 2
PRC1	Polycomb repressive complex 1
Ring1	ring finger protein 1
Runx1	runt-related transcription factor 1
Shh	sonic hedgehog
Sox2	SRY (sex-determining region Y)-box 2
Suz12	suppressor of zeste 12 homologue
Tbx5	T-box 5
TNF	tumour necrosis factor
Utx	ubiquitously transcribed tetratricopeptide repeat, X chromosome

shown to be required for normal fin regeneration in the zebrafish, although these factors are not expressed at high levels or upregulated during regeneration (Christen *et al*, 2010).

Important differences seem to exist between these processes. First, regenerating cells undergo limited dedifferentiation and preserve a memory of their origin, whereas the cell-of-origin memory needs to be completely erased to achieve full pluripotency. Second, during regeneration, specific embryonic genes that are not expressed in pluripotent or reprogrammed cells are activated. Furthermore, the low efficiency of reprogramming suggests that the pluripotency genes are immersed in a refractory chromatin environment in adult cells, which prevents their reactivation. It is therefore unlikely that these genes can be reactivated in response to injury signals during regeneration. Overall, the limited cellular plasticity that characterizes regeneration is different from the dramatic epigenetic changes that take place during reprogramming to pluripotency. However, partly reprogrammed cells that can self-renew and preserve a memory of their cell-of-origin (Sridharan *et al*, 2009) might be closer to the cellular-plasticity status achieved during regeneration. The molecular events that cause some cells to remain trapped in a partly reprogrammed state after transduction with the four factors are probably stochastic and not yet fully understood.

Lessons for regenerative medicine

Regeneration through dedifferentiation does not seem to be common in mammals. Instead, mammalian tissue regeneration seems to occur through the activation of undifferentiated multipotent precursor cells. The reason that evolution has promoted stem-cell approaches instead of dedifferentiation mechanisms remains unknown. The most-intuitive explanation is that re-growing more complex structures—such as limbs—is an excessive challenge. Alternatively, preventing the activation of embryonic programmes in differentiated cells might be a safety mechanism by which to preserve cell identity and protect against tumorigenesis. This could have been achieved by the complete resolution of bivalent domains at genes that promote dedifferentiation or initiation of embryonic programmes, making them refractory to stimulation by injury signals.

Two approaches to the replacement of damaged cells in regenerative medicine emerge from the above studies. One involves the transdifferentiation or reprogramming of cells *in vitro*. This strategy offers some advantages, such as the possibility of obtaining large numbers of cells or correcting genetic abnormalities. However, *in vitro* manipulation of cells can cause an accumulation of genetic aberrations (Laurent *et al*, 2011; Mayshar *et al*, 2010) and compromise the proper engraftment of transplanted cells in the recipient tissue. *In vivo* engineered transdifferentiation of one cell type into another could perhaps solve this problem, but might not be feasible for all tissues.

These strategies imply changing cell identity, a process that needs to overcome the chromatin barrier that preserves it (Sidebar A). The following strategies have been used to achieve this goal: (i) fusion of transcription factors with strong transactivation domains, such as VP16, that help to recruit remodelling complexes to activate target genes (Cao *et al*, 2004); (ii) treatment with inhibitors of histone deacetylases, methyltransferases and DNA methyltransferases (Feng *et al*, 2009); (iii) combining the overexpression of transcription factors with crucial chromatin remodellers (Singhal *et al*, 2010).

Importantly, incomplete reprogramming of the epigenome could lead to aberrant behaviour of cells, such as deregulation of proliferation or premature senescence. Understanding the way in which plasticity and identity are controlled at the molecular level will therefore help to improve reprogramming strategies, and to understand the potential risks that these therapies might involve.

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