Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration

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Abstract | The ultimate goal of regenerative medicine is to replace lost or damaged cells. This can potentially be accomplished using the processes of dedifferentiation, transdifferentiation or reprogramming. Recent advances have shown that the addition of a group of genes can not only restore pluripotency in a fully differentiated cell state (reprogramming) but can also induce the cell to proliferate (dedifferentiation) or even switch to another cell type (transdifferentiation). Current research aims to understand how these processes work and to eventually harness them for use in regenerative medicine.

Pluripotency
The ability of a cell to give rise to all cells of the embryo. Cells of the inner cell mass and its derivative embryonic stem cells are pluripotent.

Totipotent
Pertaining to the ability of a cell to give rise to all cells of an organism, including embryonic and extra-embryonic tissues. Zygotes are totipotent.

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Humans have a limited capacity to regenerate and restore their tissues and organs (such as blood and the liver). This can be achieved either through the activation of somatic stem cells located in a niche (as occurs, for example, with blood) (BOX 1) or by inducing differentiated cells to proliferate (as occurs, for example, in the liver). By contrast, some other vertebrate species have extensive regenerative capacities that, in certain cases, stretch as far as replacing complete limbs[1]. By studying these regenerative phenomena, researchers are beginning to uncover the cellular and molecular mechanisms at work that could be used to develop regenerative strategies for humans.

One of the mechanisms associated with natural regeneration is dedifferentiation, which involves a terminally differentiated cell reverting back to a less-differentiated stage from within its own lineage. This process allows the cell to proliferate again before redifferentiating, leading to the replacement of those cells that have been lost (FIG. 1).

Transdifferentiation is another naturally occurring mechanism that was first observed in the regenerating lens of the newt over 100 years ago[2]. This process takes dedifferentiation a step further and sees cells regressing to a point where they can switch lineages, allowing them to differentiate into another cell type (FIG. 1).

Reprogramming aims to induce differentiated cells into reverting to pluripotency. From here, they can differentiate into almost any cell type (FIG. 1). Although reprogramming occurs naturally during fertilization to produce totipotent cells that can differentiate into any cell type[3], it has not yet formally been shown to be a genuine regenerative response. Furthermore, reprogramming sidesteps the necessity of using embryos for regenerative therapies by using differentiated cells taken from a patient. From a clinical perspective, this comes with the additional bonus of circumventing the immunological problems associated with engraftment (such as transplant rejection and graft-versus-host disease).

In this Review, we highlight the current understanding of how these processes work, providing, wherever possible, natural and experimental examples. By discussing the similarities and differences between these models, we hope to highlight not only that lessons learnt from one mechanism can be applied to the others but also that each process has distinct advantages, a fact that should be kept in mind if we are to realize the full potential of these processes for regenerative medicine.

Natural dedifferentiation

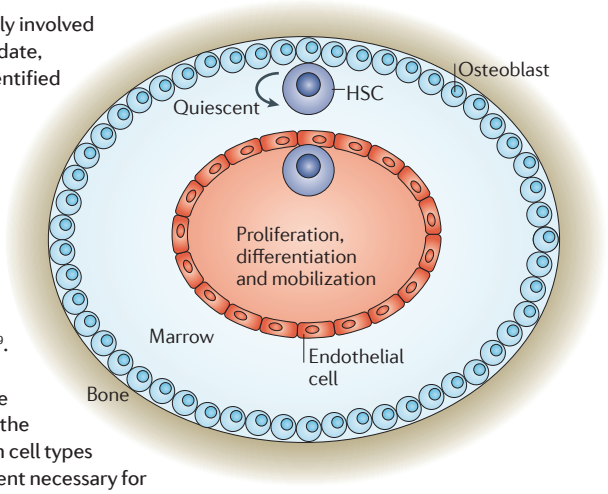
Several non-mammalian vertebrate species possess a remarkable capacity to regenerate. In many cases, this process involves the dedifferentiation of mature cells.

Dedifferentiation and heart regeneration. Zebrafish can fully regenerate their heart following amputation of up to 20% of the ventricle[4,5]. During this process, differentiated cardiomyocytes that are still present in the heart dedifferentiate and proliferate to regenerate the missing tissue[6,7] (FIG. 2). The sarcomeric contractile apparatus is a prominent feature of mature cardiomyocytes and occupies a large proportion of the cell. Because of this, the sarcomere is not conducive for cell division, as it physically impedes cytokinesis. Accordingly, during the dedifferentiation process, the sarcomere is disassembled,

Box 1 | **Regeneration and the stem cell niche**

Somatic stem cells (SSCs) present in adults are primarily involved in the renewal and regeneration of specific tissues. To date, numerous different populations of SSCs have been identified in a range of tissues such as blood, skin, intestine and neural tissue. SSCs normally reside in a niche within a specific tissue, and this provides the necessary microenvironment for SSC maintenance as well as a platform from which SSCs can be mobilized to mount a regenerative response.

The best-characterized SSCs are haematopoietic stem cells (HSCs). These possess an enormous regenerative capacity; in fact, a single HSC can repopulate the entire lymphohaematopoietic system[89]. At present, two HSC niches have been observed, one consisting primarily of osteoblasts at the marrow–bone interface, and one made up of endothelial cells within the microvasculature of the marrow[90] (see the figure). Both cell types have important roles in regulating the microenvironment necessary for HSC maintenance and regulation. Each niche seems to regulate HSCs in a different manner: the osteoblast niche maintains HSCs in a quiescent state[91,92] and the endothelial niche regulates proliferation, differentiation and mobilization of HSCs[93] (see the figure).



Numerous molecules have been identified that are involved in the regulation of HSCs. For example, stem cell factor (SCF) is produced by endothelial cells either as a soluble form or as a transmembrane protein[94]. SCF can bind to KIT receptor (also known as SCFR and CD117), which is present on HSCs and is involved in both HSC maintenance and guidance towards the niche[95,96]. HSCs also express Notch, which binds to its transmembrane ligand Jagged 1 on osteoblasts and thereby maintains undifferentiated HSC pools[97]. Osteoblasts and endothelial cells also secrete molecules that guide HSCs back to the niche when they mobilize into the bloodstream. For example, CXC-chemokine 12 (CXCL12; also known as SDF1) is produced by both osteoblasts and endothelial cells and binds to CXC-chemokine receptor 4 (CXCR4) on the surface of HSCs[90].

and this is accompanied by a decrease in the expression of sarcomeric genes such as ventricular myosin heavy chain (*vmhc*)[6,8].

As the cardiomyocytes disassemble their contractile apparatus, they also detach from one another and begin to express the positive cell cycle regulators polo-like kinase 1 (*plk1*), monopolar spindle 1 (*mps1*; also known as *ttk*) and *cdc2* (REFS 4,6,8). At present, little is known about the signalling pathways involved, although fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) signalling have been directly linked to regeneration. Indeed, inhibition of FGF or PDGF signalling leads to incomplete regeneration and the formation of scar tissue[9,10].

During embryonic development in many species, cardiomyocytes proliferate readily (hyperplasia) but soon after birth they switch to hypertrophic growth. Further increases in cardiac mass are, from then on, generated by an increase in cardiomyocyte size instead of number[11]. A reversal of this switch provides the most logical explanation for the regenerative response observed in zebrafish. Adult hypertrophic cardiomyocytes dedifferentiate back to the embryonic hyperplastic stage, which allows them to proliferate once more[12] (FIG. 2).

Dedifferentiation and limb regeneration. Another good example of dedifferentiation occurs during limb regeneration in the urodele amphibians. Shortly after limb amputation, cells adjacent to the wound dedifferentiate, forming a blastema that consists of undifferentiated cells that proliferate and eventually redifferentiate to create all the components of the lost limb. During the regenerative process, the tumour suppressor retinoblastoma protein (RB) plays a major part in allowing differentiated cells to re-enter the cell cycle. RB is a member of the pocket protein family and is crucial for arresting cell cycle progression by inhibiting E2F transcription factors and stabilizing the cell cycle inhibitor p27 (REF. 13). In addition, RB functions as a transcriptional cofactor and as an adaptor protein for chromatin-remodelling proteins[13]. During limb regeneration, RB is inactivated by hyperphosphorylation, which allows mature cells to dedifferentiate and subsequently enter the cell cycle[14].

For many years, the blastema was regarded as a homogenous population of progenitor cells[15]. This hypothesis proposed that cells near to the wound dedifferentiate back to a multipotent, or stem cell state, proliferate and then differentiate again to regenerate the missing limb. However, recent evidence suggests that the dedifferentiated blastema cells stay within their original lineage. This indicates that the cells either do not regress all the way to pluripotency or that if they do then they also somehow retain a memory (possibly epigenetic[16]) of their tissue of origin[17].

Dedifferentiation and Schwann cell proliferation. In mammals, Schwann cells possess a natural regenerative capacity. Following damage to the nerve that they are associated with, Schwann cells also dedifferentiate and proliferate[18]. During development, neural crest cells give rise to defined Schwann cell precursors. These then progress to form immature Schwann cells, before finally differentiating into mature myelinating or non-myelinating Schwann cells[19]. Importantly, precursors and immature Schwann cells readily proliferate, whereas mature cells do not. When

Hyperplasia
Growth that is due to an increase in the number of cells.

Hypertrophic growth
Growth that is due to an increase in the size of cells.

Multipotent
Pertaining to the ability of a cell to give rise to different cell types of a given cell lineage. Most adult stem cells, such as gut stem cells, skin stem cells, haematopoietic stem cells and neural stem cells, are multipotent.

Schwann cell
A cell type that wraps around the axons of peripheral nerves and forms the insulating myelin sheath.

Neural crest cell
A cell type that migrates during neurulation and forms most of the peripheral nervous system (and many other structures) in the embryo.

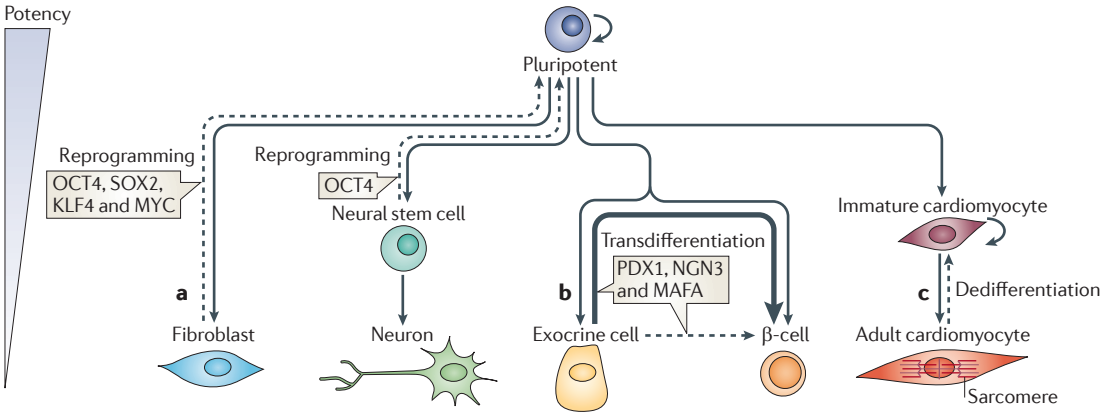


Figure 1 | **Overview of reprogramming, transdifferentiation and dedifferentiation.** Pluripotent cells are capable of differentiating (solid arrows) down any given lineage to give rise to a range of different cell types. **a** | Reprogramming (dashed arrow) fibroblasts to regress back to pluripotency requires the expression of the transcription factors OCT4 (also known as POU5F1), SOX2, Krüppel-like factor 4 (KLF4) and MYC. By contrast, neural stem cells can be effectively reprogrammed using just OCT4. **b** | During transdifferentiation, cells switch lineages to create another cell type. For example, pancreatic exocrine cells can be induced to transdifferentiate into β-cells by expressing the transcription factors pancreas and duodenum homeobox 1 (PDX1), neurogenin 3 (NGN3) and MAFA. It is unclear how exocrine cells transdifferentiate into β-cells. This may occur either directly (dashed arrow) or it could involve a dedifferentiation step (bold arrow). **c** | Dedifferentiation refers to a regression of a mature cell within its own lineage, which, in many cases, allows it to proliferate (curved arrow). Mature zebrafish cardiomyocytes proliferate during heart regeneration. This involves a dedifferentiation step (dashed arrow) that may facilitate this process.

mature Schwann cells lose contact with the axon that they are myelinating, they dedifferentiate and, before proliferating, they begin to re-express genes that are associated with immature Schwann cells[18]. Notch signalling has a prominent role during regeneration by not only inducing the proliferation of immature Schwann cells but also promoting the demyelination of mature Schwann cells, which is a crucial step of dedifferentiation[20]. Another key component is the transcription factor JUN, which has been shown to negatively regulate myelination[19]. Following nerve injury, the expression of JUN increases as Schwann cells dedifferentiate; accordingly, if JUN is suppressed, dedifferentiation is inhibited[19].

Dedifferentiation and the cell cycle. The examples of dedifferentiation that we have discussed so far suggest that dedifferentiation is intricately linked to the cell cycle. However, re-entry into the cell cycle does not seem to be necessary following dedifferentiation. Recent evidence indicates that RB not only regulates cell cycle arrest but also might have an active role in maintaining the differentiated status of a cell. *Drosophila melanogaster* is an attractive model to study RB function, as eye development follows a precisely coordinated pattern of proliferation and differentiation[21]. Mutant *D. melanogaster* with inactive forms of both RB and Hippo (a member of the STE20 family of protein kinases)[22] maintain a normal neuronal differentiation programme. However, the cells fail to retain their differentiated status; they dedifferentiate to an earlier eye-precursor stage and show unrestricted proliferation. Importantly, blocking proliferation does not stop dedifferentiation from occurring. It therefore seems that dedifferentiation and the cell cycle are distinct from one another and that RB also has an important role in maintaining the differentiated status of a cell[23].

Further support for this conclusion came from a study showing that Schwann cell dedifferentiation can also be uncoupled from proliferation. Cyclic AMP is involved in promoting Schwann cell myelination. In particular, cAMP removal leads to Schwann cell dedifferentiation *in vitro* without any subsequent proliferation. In fact, Schwann cells can be induced to repeatedly differentiate and dedifferentiate without entering the cell cycle by repetitively adding or removing cAMP[24].

Experimental dedifferentiation

Inducing dedifferentiation is a logical strategy to promote regeneration in mammalian tissues that lack this ability. Studies have indicated cell dedifferentiation occurs during tissue regeneration both *in vitro* and *in vivo*.

Muscle proliferation. Although newt myotubes readily dedifferentiate and proliferate, mouse myotubes do not. So do mouse myotubes lack some intracellular components? Or are they missing extrinsic cues? To address this, researchers reasoned that certain components that are present during newt limb regeneration may be able to induce mammalian cells to dedifferentiate. Treating mouse myotubes with an extract isolated from regenerating newt limbs reduces the expression of myoblast determination (*MyoD*) and myogenin, two genes that are associated with muscle differentiation, and subsequently allows the myotubes to dedifferentiate and proliferate[25]. As discussed above, hyperphosphorylation of RB has a crucial role during limb regeneration[14]. However, inactivation of only RB in mammalian muscle cells does not lead to cell cycle re-entry[26]. This is because the tumour suppressor ARF (alternate reading frame) — which is encoded by the *Ink4a* locus and is frequently mutated in many cancers — can compensate for RB inactivation by activating

Myotube
An elongated multinucleate cell (with three or more nuclei) that contains some peripherally located myofibrils. Myotubes are formed *in vivo* or *in vitro* by the fusion of myoblasts; they eventually develop into mature muscle fibres that have peripherally located nuclei and most of their cytoplasm filled with myofibrils.

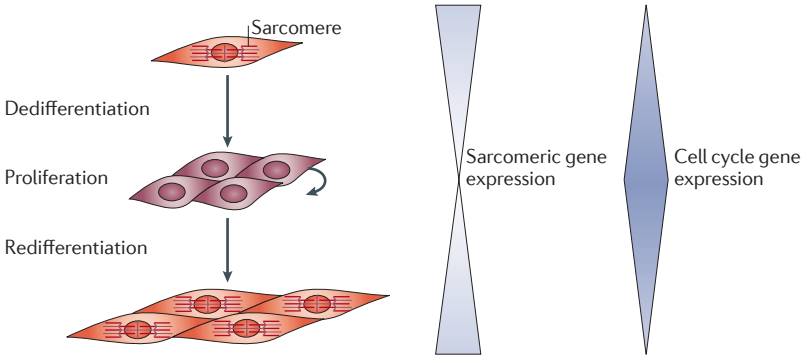


Figure 2 | **Natural dedifferentiation during zebrafish heart regeneration.** Following amputation of up to 20% of the ventricle, zebrafish cardiomyocytes begin to dedifferentiate and proliferate. As cardiomyocytes dedifferentiate, they disassemble their sarcomeric contractile apparatus, and this is accompanied by a downregulation of sarcomeric genes such as ventricular myosin heavy chain (*vmhc*). The cardiomyocytes also begin to express positive regulators of the cell cycle, such as polo-like kinase 1 (*plk1*) and monopolar spindle 1 (*mps1*; also known as *ttk*), allowing them to proliferate and regenerate the missing tissue. Finally, the cells redifferentiate back to adult cardiomyocytes.

p53-mediated growth arrest[27]. Indeed, inactivation of both RB and ARF can induce mammalian muscle cells to dedifferentiate and proliferate. Dedifferentiatiation of the cells is accompanied by a reduced expression of myogenin (a transcription factor that drives muscle cell differentiation) and myosin heavy chain (MHC; a component of the sarcomere that is necessary for muscle contraction) and a concomitant increase in the expression of the positive cell cycle regulators cyclin D1 and cyclin E. It is interesting, then, to note that ARF is not present in many regenerating vertebrates, which may partly explain why mammalian cells have lost the ability to regenerate[28].

Cardiomyocyte proliferation. The extensive research into promoting mammalian cardiomyocytes to proliferate provides some insights into the role of dedifferentiation in this process. Studies on the role of RB indicate that dedifferentiation of mature cardiomyocytes can facilitate their proliferation. Specifically, during the hyperplastic phase of cardiac development, RB is absent and appears later in terminally differentiated hypertrophic cells[29]. Mice lacking both RB and RB-like 2 (RBL2; also known as p130), which can compensate for loss of RB, show an increased heart size and enhanced cardiomyocyte proliferation[30]. As discussed above, RB seems to be able to maintain the differentiated status of a cell, which suggests that the increased cardiomyocyte proliferation may be due, in part, to a failure to maintain differentiation and a concomitant persistence of hyperplastic growth.

Further support for a requirement of dedifferentiation in cardiomyocyte proliferation comes from the study of p38 mitogen-activated protein kinase (p38 MAPK; also known as MAPK14) and FGF1. p38 MAPK can induce the cell cycle arrest that normally precedes muscle differentiation by inhibiting the RAF family proteins (hereafter referred to as RAF) and extracellular signal-regulated kinase (ERK) pathway[31]. In cardiomyocytes, p38 MAPK activity is inversely correlated with cardiomyocyte proliferation, low activity is associated with hyperplasia and high activity occurs in terminally differentiated cardiomyocytes. By contrast, FGF1 can induce mature cardiomyocytes to express fetal genes, including atrial natriuretic peptide (ANP; also known as ANF) and brain natriuretic peptide (BNP; also known as NPPB), which are expressed at much higher levels during fetal development, along with the ETS transcription factor polyomavirus enhancer activator 3 (PEA3; also known as ETV4). Together, these findings suggest that a combination of FGF1 stimulation and p38 MAPK inhibition can induce mammalian cardiomyocytes to dedifferentiate and to disassemble their contractile apparatus before proliferating[32,33].

However, there is also evidence suggesting that dedifferentiation is not necessary for cardiomyocyte proliferation. Neuregulin is an extracellular ligand that binds to ERB receptors and plays a prominent part in cardiomyocyte generation during development[34–36]. Treating differentiated cardiomyocytes *in vivo* with neuregulin induces them to re-enter the cell cycle and proliferate. Although this also involves the disassembly of the contractile apparatus, the authors of this study[37] found no evidence for dedifferentiation. So, whether mammalian cardiomyocytes need to dedifferentiate in order to proliferate is still unclear.

Natural transdifferentiation

Another option for regenerating lost or damaged tissue is to convert an existing differentiated cell into the required cell type. Natural transdifferentiation occurs in two steps: first, the cell dedifferentiates; and second, the natural developmental programme is activated, allowing the cell to differentiate into the new lineage (FIG. 3).

The regenerating lens of the newt perfectly illustrates naturally occurring transdifferentiation[2,38]. When the lens is removed, pigmented epithelial cells (PECs) from the dorsal iris transdifferentiate and regenerate the missing tissue. To achieve this, PECs must first dedifferentiate and proliferate to create a new lens vesicle, and then differentiate into the mature cells of the lens (FIG. 3a). Soon after lentectomy, PECs lose their pigmentation and change shape; at the same time, RB is inactivated through hyperphosphorylation, allowing the cells to dedifferentiate and re-enter the cell cycle[39]. Microarray analyses have revealed that, during this time, PECs upregulate cancer- and apoptosis-related genes, along with epigenetic modifiers such as histone deacetylases and members of the Jumonji family of histone demethylases[40].

PECs must enter the cell cycle and proliferate to regenerate the missing tissue, but this is not an absolute requirement for transdifferentiation to occur. Indeed, blocking the cell cycle does not stop PECs from transdifferentiating and forming a new lens vesicle[41]. Thus, dedifferentiation, which may facilitate cell cycle entry and subsequent proliferation, is the main requirement in the transdifferentiation process. Once the new lens vesicle has been formed, the cells then differentiate by expressing FGFs and the developmental lens genes paired box 6 (*pax6*) and prospero homeobox 1 (*prox1*), before finally expressing crystallins to form mature lens cell[38].

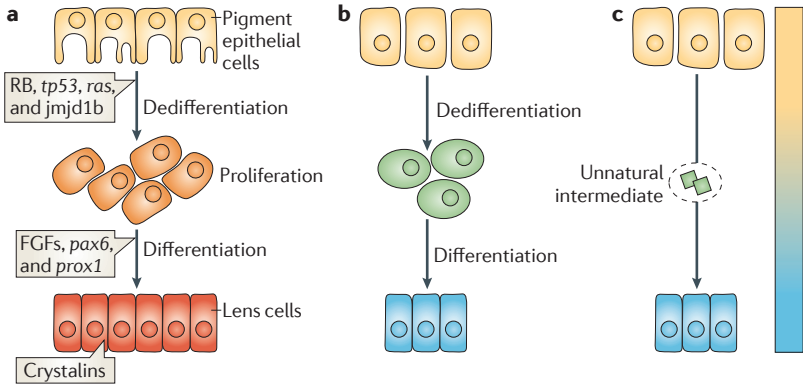


Figure 3 | **Natural and artificial transdifferentiation. a** | Newt lens regeneration. Pigmented epithelial cells (PECs) dedifferentiate, losing their pigment and changing their morphology, before entering a new lineage and differentiating into mature lens cells. During this process, retinoblastoma protein (RB) is inactivated by hyperphosphorylation. Microarray analysis of dedifferentiating PECs indicates that dedifferentiation is also accompanied by an increase in the expression of cancer-related genes such as *tp53* and *ras* and also epigenetic modifiers, such as histone deacetylases (not shown) and Jumonji family demethylases (for example, Jumonji domain-containing 1B (jmjd1b)). During the final stages of regeneration, the natural lens developmental programme is reactivated and the cells begin to express fibroblast growth factors (FGFs), paired box 6 (*pax6*) and prospero homeobox 1 (*prox1*), followed by crystallins as they reach maturity. **b**,**c** | Schematic representations of the two proposed transdifferentiation models. The first model proposes that, to transdifferentiate, a cell must first undergo dedifferentiation to a precursor stage before it can enter the new lineage and subsequently differentiate (**b**). In the second model, cells directly transdifferentiate to form the new cells, in some cases passing through an unnatural intermediate phase in which two genetic programmes are active at the same time (indicated by the gradient on the right-hand side) (**c**).

Experimental transdifferentiation

Although natural transdifferentiation involves a stepwise dedifferentiation of the primary cell to form an intermediate cell type that can then differentiate into the new lineage (FIG. 3b), it may also be possible to directly convert one cell type into another through a simultaneous downregulation of one genetic programme and a concomitant upregulation of the new genetic programme (FIG. 3c). Although this does not seem to occur naturally, it has been observed during experimental induction of transdifferentiation.

B cells to macrophages. The experimental transdifferentiation of B cells into macrophages using the transcription factors CCAAT-enhancer-binding protein-α (CEBPα) and CEBPβ provides a good example of this second mechanism[42]. During development, the differentiation of B cells from haematopoietic progenitors is initiated by the transcription factors E2A and early B cell factor (EBF). These, in turn, induce the expression of the B cell commitment factor PAX5, which subsequently upregulates many B cell-specific genes. Macrophage commitment, by contrast, is primarily induced by CEBPα and CEBPβ. However, commitment to both cell types requires the transcription factor PU.1 (also known as SPI1); indeed, PU.1-deficient mice have no B cells or macrophages[43] (TABLE 1).

CEBPα and CEBPβ are thought to induce transdifferentiation by simultaneously downregulating B cell-specific genes (such as *Pax5*), while at the same time co-activating macrophage-specific genes. En route to becoming macrophages, the B cells pass through an unnatural intermediate phase in which they express low levels of B cell-specific genes (such as *Cd19*) and macrophage-specific genes (such as *Mac1*)[42] (FIG. 3c). Although this process does not seem to involve an initial dedifferentiation step, it should be noted that deletion of *Pax5* in B cells causes them to dedifferentiate[44].

Pancreatic cell transdifferentiation. Pancreas and duodenum homeobox 1 (PDX1) has a pivotal role in the development of the pancreas (*Pdx1*-knockout mice fail to develop this organ[45]), but in adults its expression is restricted to insulin-producing β-cells[46]. However, experimentally overexpressing PDX1 can also drive the transdifferentiation of adult hepatocytes into pancreatic cells (TABLE 1). PDX1 first induces dedifferentiation by specifically repressing the hepatic transcription factor CEBPβ, leading to reduced expression of mature hepatocyte genes, such as albumin, alcohol dehydrogenase 1β and glucose-6-phosphatase, and increased expression of α-fetoprotein (a marker of immature hepatic cells)[46]. Importantly, dedifferentiation has to occur before PDX1 can initiate the pancreatic gene programme and induce the dedifferentiated cells to enter the new lineage and subsequently differentiate into hepatocytes[46].

Pancreatic exocrine cells have also been successfully transdifferentiated into insulin-producing β-cells by expressing a range of transcription factors[47]. This provides a basic strategy that could be used for many different cell types. By methodically testing different combinations of genes associated with β-cell development, three genes (*Pdx1*, neurogenin 3 (*Ngn3*) and *Mafa*) were found to be capable of inducing transdifferentiation (TABLE 1). Although the molecular mechanism is not yet fully understood, insights can been obtained from analysing how these genes operate during pancreatic development. *Pdx1* is initially required to specify the pancreatic lineage. This is followed by a transient expression of *Ngn3*, which initiates β-cell differentiation[48]. Subsequently, *Mafa* and another wave of *Pdx1* expression drive insulin production in mature β-cells[49,50]. Thus, the natural developmental programme places these three genes in a specific hierarchy. This could suggest that exocrine cells are induced to dedifferentiate by *Pdx1* before all three genes induce the natural β-cell developmental programme (FIG. 3b).

Another possibility is that the exocrine cells transdifferentiate into β-cells by going through an unnatural intermediate, similarly to B cells (FIG. 3c). This seems to be the case in a recently described β-cell regeneration model. Near complete destruction of β-cells *in vivo* forces pancreatic α-cells to transdifferentiate to replace them[51]. During this process, intermediate cells are found that produce both glucagon (normally produced by α-cells) and insulin (a product of β-cells). Although this seems to be a direct conversion, it should be stressed that α-cells and β-cells are functionally similar and share several transcription factors. Thus, a complete dedifferentiation of the α-cells may not in fact be a prerequisite for their transdifferentiation into β-cells.

β-cell
A cell type in the pancreas, found in the islets of Langerhans, that produces insulin.

Pancreatic exocrine cell
A pancreatic cell type that is responsible for the secretion of bicarbonate ions and digestive enzymes.

Neuronal transdifferentiation. Using a similar strategy, it is also possible to transdifferentiate mouse fibroblasts into functional neurons using just three transcription factors: achaete–scute homologue 1 (ASCL1), brain-specific homeobox and POU domain 2 (BRN2; also known as POU3F2) and myelin transcription factor 1-like (MYT1L)[52] (TABLE 1). Individual analysis reveals that, of these, only ASCL1 can induce an immature neuronal phenotype in fibroblasts. The exact molecular mechanism of transdifferentiation in this case is unclear, as there are no sufficient gene expression data. However, ASCL1 seems to be the main regulator because it can induce fibroblasts to transdifferentiate into immature neurons. After these immature cells have entered the neuronal lineage, the developmental gene programme seems to come into play. This involves the interaction of BRN2 with ASCL1, which pushes immature cells further along the neuronal lineage before MYT1L is finally required to generate fully differentiated mature neurons[52,53]. It is interesting that, as with β-cells, the genes used to induce neuronal transdifferentiation have a particular hierarchy.

Fibroblasts to cardiomyocytes. Fibroblasts have also been transdifferentiated into cardiomyocytes using a pool of 13 transcription and epigenetic remodelling factors known to produce severe cardiac phenotypes in knockout mice. Subsequently, by serially removing factors, only GATA4, myocyte-specific enhancer factor 2C (MEF2C) and T-box 5 (TBX5) were found

Table 1 | **Genes used to induce dedifferentiation, transdifferentiation or reprogramming**

Gene symbol*	Class	Role *in vivo*	Mouse knockout phenotype
Arf (*Cdkn2a*)	Protein kinase inhibitor	Negative regulator of proliferation	Increased tumorigenesis
Ascl1	Transcription factor	Neural lineage specification	Impaired development of various brain centres; neonatal lethality
Baf60c (*Smarcd3*)	Chromatin modulator	Neuron differentiation	Defective cardiogenesis and somitogenesis
Bcl11b	Transcription factor	Fetal thymocyte development and survival	Prenatal and perinatal lethality; haematopoietic defects
Brn2 (*Pou3f2*)	Transcription factor	Neuroectoderm specification	Perinatal lethality
Cebpa	Transcription factor	Broad target range	Neonatal lethality; multi-organ defects
Cebpb	Transcription factor	Immune and inflammatory response; brown fat specification	High neonatal hypoglycaemia and mortality
Fgf1	Growth factor	Angiogenic	Normal
Gata4	Transcription factor	Heart tube and foregut formation	Lethal; ventral defects
Klf4	Transcription factor	Differentiation of epithalial cells	Perinatal death owing to skin defects
Lin28	Transcription factor	Suppressor of microRNA biogenesis	Unknown
Mafa	Transcription factor	Activates insulin gene expression	Diabetes and pancreatic islet abnormalities
Mef2c	Transcription factor	Controls cardiac morphogenesis and myogenesis	Prenatal death and cardiovascular abnormalities
Myc	Transcription factor	Broad action on cell cycle and growth	Prenatal lethality and growth defects
Myt1l	Transcription factor	Pan-neural transcription factor with roles in neuronal differentiation	Unknown
Nanog	Transcription factor	Imposes pluripotency on embryonic stem cells and prevents their differentiation	Early embryonic death
Ngn3	Transcription factor	Neurogenesis and pancreatic endocrine cells specification	Deficiency of endocrine cells and insulin-producing cells; postnatal diabetes
p38 mapk (*Mapk14*)	Protein kinase	Inflammation and response to stress	Embryonic to perinatal lethal with multi-system defects
Pdx1	Transcription factor	Specifies early pancreatic epithelium	Postnatal lethality and abnormal pancreatic and liver development
Oct4	Transcription factor	Crucial for early embryogenesis and for embryonic stem cell pluripotency	Peri-implantation lethality; failure to develop the inner cell mass
Pu.1 (*Spi1*)	Transcription factor	Lymphoid-specific enhancer	Postnatal lethality and haematopoietic defects
Rb1	Transcription factor and chromatin modulator	Key regulator of entry into cell division	Prenatal lethality and neuronal and haematopoietic defects
Tbx5	Transcription factor	Mesoderm differentiation	Prenatal lethality and cardiovascular defects

Arf, alternate reading frame; *Ascl1*, achaete–scute homologue 1; *Baf60c*, BRG1-associated factor 60C; *Bcl11b*, B cell lymphoma 11B; *Brn2*, brain-specific homeobox and POU domain protein 2; *Cebp*, CCAAT-enhancer-binding protein; *Fgf1*, fibroblast growth factor 1; *Klf4*, Krüppel-like factor 4; *mapk*, mitogen-activated protein kinase; *Mef2c*, myocyte-specific enhancer factor 2C; *Myt1l*, myelin transcription factor 1-like; *Ngn3*, neurogenin 3; *Pdx1*, pancreas and duodenum homeobox 1; *Rb1*, retinoblastoma protein; *Tbx5*, T-box 5. *Alternative names are provided in brackets.

to be capable of inducing the switch from fibroblast to cardiomyocyte[54]. There is much evidence showing that all three factors interact with one another to drive the genetic programme regulating cardiomyocyte differentiation[55–57]. Of the three, GATA4 may initiate the whole process by relaxing chromatin at key cardiogenic loci, thus facilitating access for the other factors access[58]. These findings offer a new regenerative strategy, as fibroblasts present in the heart could be induced to transdifferentiate *in vivo* and thus replace cardiomyocytes lost to damage or disease.

Reprogramming

Reprogramming a fully differentiated cell induces it to regress all the way back to its pluripotent beginnings, with the potential to differentiate into any other cell type. How this has been achieved by a range of different approaches was recently reviewed in REF. 59, so we concentrate on direct reprogramming by the introduction of transcription factors, pioneered by Yamanaka's group in 2006 (REF. 60).

The core transcription factors. If one imagines a pluripotent cell teetering at the top of a mountain ready to differentiate down any number of lineages if given the slightest push, one can also imagine that maintaining the cell in this poised state requires the concerted effort of an extensive array of genes and cellular processes (BOX 1). Bearing this in mind, we focus on the three core transcription factors (OCT4 (also known as POU5F1), SOX2 and NANOG) that seem to mastermind the whole process. OCT4 has an essential role in the development and maintenance of pluripotency by activating or repressing numerous genes[61,62]. In fact, cells lacking functional OCT4 can no longer sustain this key attribute[63]. SOX2 is not restricted to pluripotent cells, as it is also the earliest definitive marker of the neural plate[64]. Alone, it is unable to bind stably to DNA and thus requires a cofactor[65]; in particular, SOX2 readily forms heterodimers with OCT4. Although SOX2 has been implicated in the self-renewal of stem cells, one important function is to maintain OCT4 expression at the appropriate levels needed to maintain pluripotency[66,67]. During development, NANOG is expressed by the pluripotent cells of the inner cell mass (ICM) and, accordingly, embryos that lack this gene fail to develop the ICM[68]. However, if NANOG is removed from cells that have already attained pluripotency, they still retain many stem cell attributes[69]. Thus, it seems that NANOG's major role is in the acquisition of pluripotency.

Cooperative action. In their primary role as key regulators of pluripotency, OCT4, SOX2 and NANOG cooperate on many levels to maintain this attribute. Not only can they directly activate and repress transcription but also they can control gene expression and the cell cycle by regulating epigenetic modifiers and microRNAs[70,71] (BOX 2). All three transcription factors can also self-regulate their transcription levels by binding to sites within their own promoters, which may well be the key behind maintaining pluripotency[72]. In addition, OCT4 and SOX2 directly regulate the expression of NANOG, which could explain why it is not one of the essential transcription factors required for generating induced pluripotent stem (iPS) cells[73]. Much work has been done to elucidate the targets of these core transcription factors, and this work has shown that all three can activate a wide range of pluripotency-associated genes and repress many genes associated with differentiation[61,62,74].

Inducing pluripotency

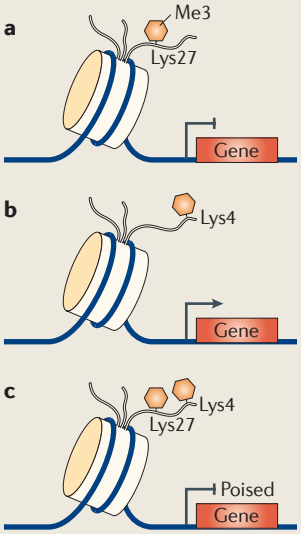
Although major inroads have been made into understanding the mechanisms of reprogramming[75], our knowledge of this process is still in its infancy. Reprogramming may seem completely random, with genes indiscriminately activated and repressed until a combination that promotes pluripotency is established; however, there is increasing evidence to suggest that this is not entirely the case and that there is some order among the chaos.

Which factors? Since the initial finding that fibroblasts can be reprogrammed to pluripotency with the four transcription factors OCT4, SOX2, Krüppel-like factor 4 (KLF4) and MYC[60,76], it has become apparent that this specific combination does not have to be strictly adhered to. For example, fibroblasts can actually be reprogrammed without MYC[77], and cord blood cells can be readily reprogrammed with just OCT4 and SOX2 (REF. 78). To date, the cells most amenable to reprogramming have been neural stem cells, which only require OCT4 (REF. 79) (TABLE1). Why certain cell types require fewer factors remains controversial. One possibility is that less-differentiated cells are, effectively, closer to pluripotency and thus require fewer exogenous factors. Another possibility is that certain cell types already express some of these factors endogenously. The only real consensus at present is that OCT4 (either supplied exogenously or induced endogenously[80]) is indispensable for reprogramming.

Initiation event. There is increasing evidence to suggest that some kind of cellular process or event occurs before the exogenous transgenes are capable of inducing pluripotency. For instance, it takes a substantial period of time to induce pluripotency and, within any given population, individual cells will become pluripotent at different times despite expressing similar levels of exogenous genes[81]. The cell cycle seems to have an important role in reprogramming, and great reductions in latency can be achieved by increasing cell proliferation[81]. One explanation for this is that, during the process of entering the cell cycle and proliferating, naturally occurring events such as epigenetic modifications and changes in gene expression make these cells more responsive to the reprogramming process. In effect, this could make chromatin more accessible and also result in the repression of genes that are associated with maintaining the differentiated state. In any case, this initial event primes the cell, making it amenable to reprogramming (FIG. 4).

Box 2 | **Epigenetics and microRNAs**

The roles of epigenetics and microRNAs (miRNAs) in pluripotency have been reviewed elsewhere[70,75,98,99], so only a few points are summarized below.



Epigenetics

Post-translational histone modifications induce conformational changes and ultimately regulate the accessibility of the DNA coiled around them. The polycomb group (PcG) proteins assemble into two separate complexes, polycomb repressive complex 1 (PRC1) and PRC2, which catalyse the methylation of Lys residues present in histones. In particular, PRC2 catalyses the trimethylation of histone H3 at Lys27 (H3K27me3), a repressive modification that is associated with maintaining pluripotency and self-renewal (see the figure, part **a**)[100,101]. This also serves as a docking site for PRC1, which can subsequently induce chromatin compaction[102]. By contrast, the trithorax group (TrxG) proteins catalyse the H3K4me3 histone modification, which is associated with gene activation (see the figure, part **b**)[103]. A hallmark of embryonic stem cells is the presence of bivalent histone modifications, H3K4me3 and H3K27me3, which repress many differentiation genes but leave them 'poised' for activation (see the figure, part **c**)[104,105].

Methylation is mediated by DNA methyltransferases (DNMTs), which catalyse the addition of Met groups to Cys residues in CpG islands (typically abundant in the promoter regions of many mammalian genes). Methylation can either impede the binding of transcription factors or act as a recognition site for recruiting repressive epigenetic factors. In pluripotent cells, DNA methylation serves to repress differentiation and also to silence the exogenous transgenes[106].

Demethylation can be mediated by members of the Jumonji family. Both Jumonji domain-containing 1A (JMJD1A) and JMJD2C are positively regulated by OCT4 (also known as POU5F1) and induce the expression of genes associated with pluripotency and self-renewal, including NANOG[72,107].

miRNAs

miRNAs act post-transcriptionally to either target specific mRNAs for degradation or suppress their translation[108]. Increasing evidence suggests that miRNAs also have a significant role in regulating pluripotency. For example, embryonic stem cells lacking either of the key miRNA-processing proteins Dicer or DiGeorge syndrome critical region 8 homologue (DGCR8) show defects in proliferation and differentiation, two processes that are essential for the maintenance of self-renewal and pluripotency[99]. Furthermore, OCT4, SOX2 and NANOG occupy the promoters of many miRNAs that are preferentially expressed by pluripotent cells. In particular, the mir290 cluster regulates the expression of the methyltransferases DNMT3a and DNMT3b[109].

The intermediate stages. Once a cell has been primed, the acquisition of pluripotency does not occur immediately. As more and more events, such as epigenetic modifications, activation of stem genes and repression of differentiation genes, take place, the cell regresses back to pluripotency. But is this process as random as it seems? Do various combinations of events occur at different times in different cells? If so, this would mean that any given cell could theoretically take a completely different route back to pluripotency. Another possibility is that a certain sequence of stages must be traversed for a cell to gain pluripotency (again, however, how cells reach these stages could be completely random). This possibility is supported by the identification of intermediate or partially reprogrammed cells, which seem to be genetically similar despite originating from completely different cell types[76,82,83] (FIG. 4). In particular, stable partially reprogrammed cells only reactivate a subset of stem cell-associated genes and fail to repress many genes associated with differentiation. Although these cells have not managed to attain pluripotency, additional manipulation can coax them into completing this last step[82,84]. Further evidence for intermediate stages comes from imaging individual fibroblasts throughout the reprogramming process. This has revealed that pluripotent colonies emerge from a distinct class of small, rapidly dividing cells that appear soon after induction has begun[85]. Similar analysis of different cell types, combined with genetic and epigenetic data, should be able to determine whether this is indeed another common reprogramming intermediate.

Taken together, the identification of these intermediate stages argues against a completely random process and indicates that a particular, albeit ill-defined, sequence of stages must be traversed to attain pluripotency.

The acquisition of pluripotency. The final stage of reprogramming sees the cells attain and maintain pluripotency, switching to the endogenous programme and, in so doing, shutting down the exogenous transgenes. At this stage, NANOG becomes a crucial determinant. As already discussed, removal of NANOG from embryonic stem cells does not affect their ability to maintain pluripotency; however, during reprogramming, NANOG is essential if a cell is to attain a pluripotent status. This is demonstrated by cells lacking NANOG, which can be reprogrammed to intermediate stages but fail to develop pluripotency[68]. In this respect, it becomes apparent why NANOG is not one of the essential exogenous factors — addition of OCT4 and SOX2 is sufficient to drive endogenous NANOG expression and establish pluripotency (FIG. 4).

Finally, the endogenous pluripotency programme needs to be activated for the cell to become independent from the exogenous transgenes. This occurs late in reprogramming[86] and may involve subduing repressive mechanisms such as histone and DNA methylation, a notion that is supported by the improved reprogramming efficiency seen when these are perturbed[82,87]. Once the endogenous programme is in place, the self-regulatory mechanisms that are associated with OCT4, SOX2 and NANOG help to maintain pluripotency. Importantly, there must be a complete repression of lineage-specifying genes. Although this occurs throughout the reprogramming process, the cells will not be able to maintain pluripotency if any genes that promote differentiation are still active. This is achieved through the regulation of DNA methyltransferases by OCT4, SOX2 and NANOG, which play a crucial part in repressing lineage-specifying genes and silencing exogenous transgenes[70].

The three processes compared

All three of the processes that we have discussed so far can induce terminally differentiated cells to become highly plastic. Although many of the mechanisms involved in dedifferentiation and transdifferentiation have been elucidated, relatively little is known about what occurs

DNA methyltransferase
An enzyme that catalyses the addition of a methyl group to C or A. DNMT1 is a maintenance DNA methyltransferase, DNMT3a and DNMT3b are *de novo* DNA methyltransferases.

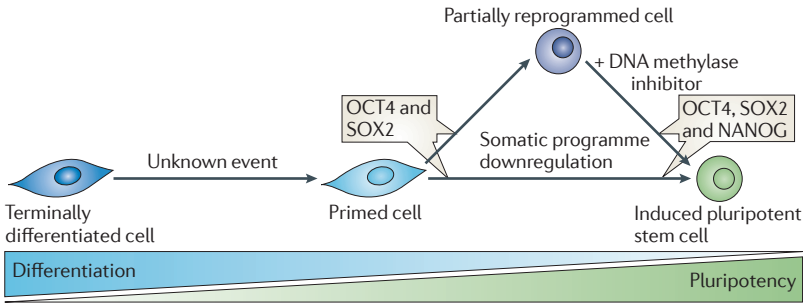


Figure 4 | **The induction of pluripotency.** Terminally differentiated cells become primed by an as-yet-unknown event. This priming event may lead to a relaxation of previously restricted chromatin, allowing the exogenous transcription factors access to previously repressed genes. Once the cell is primed, the exogenously supplied OCT4 (also known as POU5F1) and SOX2 begin the induction of pluripotency. This seems to be random, with no clear molecular milestones. As a consequence of this stochasticity, attaining pluripotency is not always guaranteed and can result in partially reprogrammed cells. Although these cannot yet be ascribed to any particular lineage, they do possess a signature gene expression pattern despite originating from completely different cell types. Further manipulation, such as inhibition of DNA methylases, can coax partially reprogrammed cells into achieving pluripotency. Finally, NANOG is also required to allow the cells to reach the pluripotent state. Once the cell has reached this point, the endogenous programme comes into play to maintain pluripotency and self-renewal.

during reprogramming. But could some of the processes involved in dedifferentiation or transdifferentiation also apply to reprogramming?

Dedifferentiation vs reprogramming. Dedifferentiation and reprogramming seem outwardly very similar, as they both induce a differentiated cell to regress. It could be argued that reprogramming is, in fact, the ultimate form of dedifferentiation, as the cells regress to a pluripotent state. Currently, the regression of a differentiated cell is the main similarity between these two processes, so could other mechanisms associated with dedifferentiation also occur during reprogramming? For example, it seems that for a cell to dedifferentiate it needs to be 'unlocked' from its terminally differentiated status before it can regress or, in many cases, enter the cell cycle[23]. During reprogramming, an initial unknown event occurs that allows the exogenous factors to induce pluripotency. Could this event be a loss of terminal differentiation or even complete dedifferentiation? Although this unknown event has been linked to proliferation[81], many cells also need to dedifferentiate to enter the cell cycle. This could allow transcription factors such as OCT4 and SOX2 to access previously restricted chromatin and to subsequently hijack the natural process and push the cells back even further. In support of this notion, mature B cells need to be dedifferentiated with either CEBPα or PAX5 before they can be reprogrammed[88]. It will be interesting to see whether a dedifferentiated cell can be reprogrammed if the cell cycle is blocked.

One major difference between these processes is the defined natural intermediate stages that are associated with dedifferentiation in what seems to be a reversal of developmental differentiation. No such defined natural intermediates have so far been identified during the reprogramming process, making it unlikely that a similar reversal has been instigated.

Transdifferentiation vs reprogramming. Dedifferentiation and transdifferentiation share many similarities. In fact, one of the transdifferentiation models requires an initial dedifferentiation step (FIG. 3b). But how do reprogramming and transdifferentiation compare? Taking into account the differentiation of iPS cells into specific lineages, reprogramming does seem outwardly similar to natural transdifferentiaton, with a dedifferentiation step that reverts the cell all the way to pluripotency before redifferentiating into a new lineage.

However, transdifferentiation between cells that are closely related, such as B cells and macrophages or α-cells and β-cells[42,51], has been proposed to occur through the simultaneous downregulation of one programme and the upregulation of another, leading to an unnatural intermediate phenotype (FIG. 3c). One could argue that unnatural intermediates can also arise through reprogramming when cells express both lineage-specifying and pluripotency genes[82]. Although this seems similar to the unnatural intermediates observed during the transdifferentiation of B cells to macrophages and α-cells to β-cells, the transdifferentiation intermediates are not stable and probably reflect how rapidly this process occurs, with the remnants of one genetic programme, in the form of mRNA and protein, still lingering as the new programme takes over. It must also be stressed that transdifferentiation occurs between distinct lineages, resulting in the formation of a differentiated cell and not, as in reprogramming, a pluripotent cell. It would seem then that it is too early to say whether reprogramming and transdifferentiation share any real common ground.

Applicability. Dedifferentiation, transdifferentiation and reprogramming could all be used for therapeutic purposes in regenerative medicine. However, one of the major differences between reprogramming and both dedifferentiation and transdifferentiation is their current *in vivo* potential. Although both dedifferentiation and transdifferentiation can be successful achieved *in vivo*, directing pluripotent cells into a new lineage is a complex process and one that has so far been successful only *in vitro*. Being able to generate new cells *in vivo* circumvents the need to transplant cells and thus negates many of the problems associated with this procedure. However, reprogramming does offer the opportunity to genetically modify cells. This opens up a wide range of possibilities such as correcting disease-inducing mutations. Clonal populations of genetically engineered iPS cells can be generated *in vitro*, ensuring that only correctly modified cells are selected for subsequent use. Furthermore, these populations can also be expanded, which is especially pertinent if the objective is to replace cells lost to damage or disease.

Although the three processes can induce significant changes in differentiated cells, each has certain advantages in terms of regenerative medicine. If the objective is to replace cells lost as a result of injury or disease, this could be achieved by reprogramming cells taken from the patient *in vitro* and then differentiating them into the correct cell type, followed by engraftment back

into the patient. A simpler *in vivo* approach would be either to induce cells to dedifferentiate and then proliferate or to induce a more abundant or less-specialized cell type to transdifferentiate into the desired cell types. If, however, the objective is to fix a disease-inducing genetic mutation, trying to transdifferentiate or dedifferentiate any of the patient's cells would not alleviate the problem, as the new cells would still contain the mutation. In this case, the only viable option would be to reprogramme cells from the patient *in vitro* then fix the damaged gene before differentiating the cells into the correct lineage and returning them back to the patient.

Conclusion and perspectives

Dedifferentiation, transdifferentiation and reprogramming have undisputedly changed the way that we view differentiated cells and how we can apply them to regenerative medicine. Rather than sitting passively and stably at the end of a multilineage valley, cells can be radically changed through the use of each of these processes. Reprogramming has certainly re-invigorated interest in the field of transdifferentation. Indeed, the original reprogramming strategy of using pooled transcription factors followed by serial subtraction has been successfully applied to transdifferentaition, as discussed above. However, one of the main differences between reprogramming and dedifferentiation or transdifferentiation is how unnatural this process seems to be. In this respect, it may be advantageous to try and adapt reprogramming strategies to follow a more natural route to pluripotency. For example, it would make sense to include a dedifferentiation step or steps at the beginning of the reprogramming strategy, allowing the transition from differentiated cell to pluripotency to occur more smoothly and efficiently. Using cells with defined lineages and dedifferentiation potentials may also help to reduce the stochasticity associated with reprogramming and also allow research to focus on the final stages of the induction process. Furthermore, the examples of transdifferentiation we discuss indicate that lineage-specific transcription factors can also induce cells to regress, which could be something to bear in mind when trying to reprogramme a specific cell type.

Currently, one of the major issues with reprogramming is safety. Specifically, the stress placed on cells during reprogramming can lead to the selection of iPS cells in which stress regulatory genes are mutated, which makes them more prone to tumour formation. Instead of trying to force a cell all the way back to pluripotency in one go, it may be prudent to try and achieve this in stages, similar to what occurs during natural dedifferentiation and transdifferentiation.

Further understanding of how these processes work will not only benefit the therapeutic use of each process individually but also could be used across the board to expand our knowledge of the three processes together.

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Competing interests statement

The authors declare competing financial interests: see web version for details.

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