Turning straw into gold: directing cell fate for regenerative medicine

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Abstract | Regenerative medicine offers the hope that cells for disease research and therapy might be created from readily available sources. To fulfil this promise, the cells available need to be converted into the desired cell types. We review two main approaches to accomplishing this goal: *in vitro* directed differentiation, which is used to push pluripotent stem cells, including embryonic stem cells or induced pluripotent stem cells, through steps similar to those that occur during embryonic development; and reprogramming (also known as transdifferentiation), in which a differentiated cell is converted directly into the cell of interest without proceeding through a pluripotent intermediate. We analyse the status of progress made using these strategies and highlight challenges that must be overcome to achieve the goal of cell-replacement therapy.

Directed differentiation
The process by which pluripotent stem cells are induced to assume a particular cell fate, through the application of specific culture conditions designed to produce cell-fate changes similar to those observed in the formation of the target cell type *in vivo*.

Reprogramming
(Also referred to as transdifferentiation.) The direct interconversion of one fully differentiated cell type to another without a pluripotent or multipotent intermediate, often achieved through transcription-factor overexpression.

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Many human diseases are caused by deficits in the quantity or functionality of particular cells. These diseases include neurodegenerative disorders, certain forms of blindness and deafness, diabetes, and some types of liver and heart disease. If it were possible to create and deliver replacement cells to patients, such diseases might be ameliorated or even cured (reviewed in REF. 1). In addition, renewable sources of cell types that cannot be readily isolated from humans or animal models would allow novel biological studies. Such cells also have promise as tools for drug discovery and toxicology testing (reviewed in REF. 2). To realize these possibilities, strategies are needed to convert plentiful cell types into the cells needed for therapeutic and research purposes.

In this Review, we describe two major strategies to direct the fate of abundant cell types into desired, but difficult to obtain, populations (FIG. 1). First, we detail a strategy referred to as directed differentiation, in which cultured pluripotent stem cells are coaxed through a series of steps that are usually designed to mimic those that produce the desired cell type *in vivo*. Because pluripotent cells can now be derived from any human patient[3], this method has the potential to produce cells that could be transferred back into the patient without any risk of immune rejection (see REF. 4 for further discussion). We discuss the various tools, particularly growth-factor and small-molecule treatments, that can be used to induce cell-fate decisions. We also highlight some of the most successful directed-differentiation protocols that have been developed so far, including those for generating neurons, hepatocytes and cardiomyocytes.

Second, we analyse a strategy termed reprogramming, which is also referred to as transdifferentiation (FIG. 1). In this strategy, one fully differentiated cell type is converted directly into another without a multipotent or pluripotent intermediate, most often by overexpression of key transcription factors[5]. Often in such strategies, fibroblasts (or some other readily available patient cell type) are selected as the starting material, theoretically permitting the generation of large quantities of patient-specific cells. Notably, recent studies have reported that fibroblasts can be reprogrammed into neurons[6], cardiomyocytes[7] and even blood-cell progenitors[8], demonstrating the wide applicability of this approach.

We also comment on some key challenges that must be overcome in both directed differentiation and reprogramming protocols, such as the relatively low efficiency of desired cell generation with these processes, the difficulty in adapting methods developed using mouse cells for use with human cells, and safety and cost concerns posed by the reagents used to direct cell fate.

We conclude by considering methods that can be used to compare various parameters in cells created *in vitro* with those of cells produced by normal development *in vivo*. Rigorously demonstrating that cells produced *in vitro* are functionally equivalent to those produced *in vivo* remains a difficult but essential element of any directed-differentiation or reprogramming procedure.

This Review does not emphasize a third possible route towards generating a desired cell type, that of transdetermination (reviewed in REFS 9,10). In this

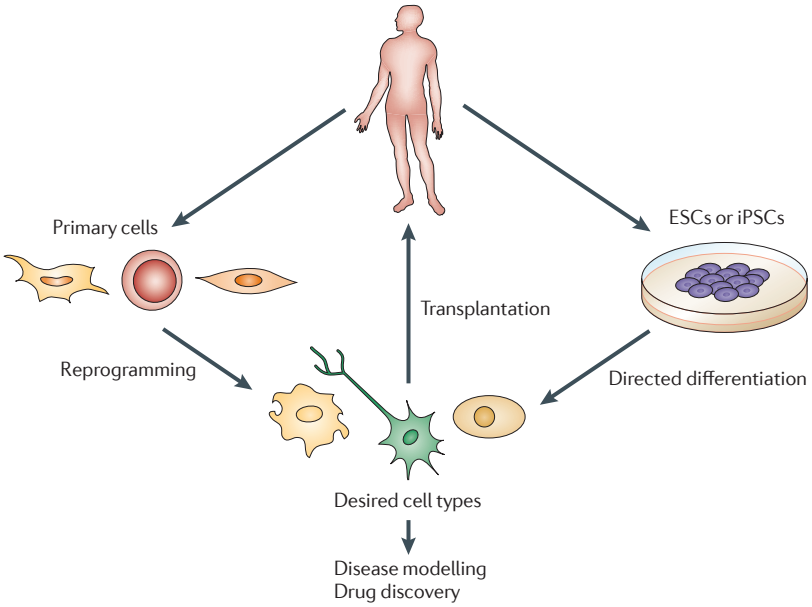


Figure 1 | **The central strategies of regenerative medicine.** This figure outlines the two main strategies for generating patient-specific cells of a desired type. Pluripotent cells to be used for regenerative medicine can be either patient-derived (induced pluripotent stem cells (iPSCs)) or non-patient-derived (either embryonic stem cells (ESCs) or iPSCs). Pluripotent cells can be differentiated *in vitro* to a desired cell state (directed differentiation, right). Alternatively, primary cells derived from a patient can be used to generate a desired cell type directly (reprogramming, left). Cells of a desired type obtained by either of these methods can then be studied *in vitro* (bottom) or used for transplantation into patients (top).

approach, an adult multipotent stem cell is induced to switch from its normal lineage to a closely related lineage and, through differentiation of its progeny, to give rise to a desired cell type. Unfortunately, adult multipotent stem cells are relatively scarce and difficult to isolate. Therefore, we believe that, although transdetermination may be a useful and relevant mechanism for tissue repair *in situ*, it is less likely to be relevant for generating large quantities of a particular cell type for *in vitro* analysis or transplantation *in vivo*.

Directed differentiation

Pluripotency is the signature characteristic of both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). The ability of these stem cells to differentiate into the many cell types of the adult organism has led to the idea that their capacity for differentiation might be controlled *in vitro* in such a way as to produce only a single, desired cell type. Nevertheless, the development of methods to control differentiation to favour the generation of one cell type over another has proved challenging.

The problem of creating a desired cell type from a pluripotent cell is usually approached using normal biological development as a guide. On the basis of knowledge of how a developing embryo (which begins as a collection of pluripotent cells) produces a particular cell type, many researchers aspire to reproduce the process *in vitro* and arrive at the same destination. The knowledge of how a cell type is produced during normal development can often be gleaned from the study of genetic mutants in which the tissue of interest fails to form correctly in the context of an embryo. Such mutations allow us to identify key transcription factors, signalling molecules or other proteins that are required for the developing organ to advance from one developmental stage to the next. Various methods, discussed below, can then be used to achieve each step *in vitro*.

Growth factors. A popular method for controlling cell fate *in vitro* is the addition to the differentiation media of recombinant growth factors that have a known role in the differentiation of the desired cell type *in vivo*. This strategy has been extremely successful in mimicking the earliest steps in embryonic development. *In vivo*, the first step in the differentiation of the pluripotent cells of the inner cell mass occurs during gastrulation, which results in the formation of the three germ layers: ectoderm, mesoderm and endoderm. As a result, a first step of many directed-differentiation protocols is the conversion of ESCs or iPSCs into cells of the germ layer that gives rise to the desired cell type.

Perhaps surprisingly, developmental studies have demonstrated that only a handful of signalling-molecule families are required to specify these three germ layers (TABLE 1). Variations in the intensity of signalling by members of the transforming growth factor-β (TGFβ) superfamily (including activin/Nodal and bone morphogenetic proteins (BMPs), reviewed in REF. 11), the WNT family[12] and the fibroblast growth factors (FGFs)[13] have been implicated in the establishment and patterning of the germ layers *in vivo*. Although the importance of these factors during embryonic development has been known for some time, the concentration of each factor to which a developing cell might be exposed in an embryo and the period of exposure have been difficult to determine. To specify the germ layers *in vitro*, each candidate factor has had to be tested at various concentrations and applied for various lengths of time. Studies of this type determined that high concentrations of the TGFβ family member activin A induce endoderm formation in both mouse[14] and human[15] ESCs. To induce mesoderm, human pluripotent cells are also exposed to activin A, albeit at much lower concentrations than those used to induce endoderm[16,17]. Some groups have also found that BMP4 is required for efficient mesoderm induction[17]. For ectoderm induction, protein antagonists of endogenous BMP and WNT signalling (Noggin[18] and DKK1 (REFS 18,19)) are used in both mouse and human cells.

Following successful induction of the desired germ layer, protein growth factors and signalling molecules, often in combination with small molecules (see next section), can push the cells along the desired differentiation pathway (FIG. 2). Many of the same signalling pathways that have a role in the specification of the germ layers have further roles in the specification of more mature cell types. By careful titration of concentration and duration of factor application, and combinatorial use of protein factors, a wide variety of cell fates can be generated using a relatively narrow panel of proteins. However,

Transdetermination
A switch in commitment from one lineage to another, closely related lineage that occurs in a multipotent stem or progenitor cell.

Embryonic stem cells
(ESCs). Pluripotent stem cells derived from the inner cell mass of a mammalian embryo.

Induced pluripotent stem cells
(iPSCs). Pluripotent stem cells derived from somatic cells by reprogramming.

Ectoderm
One of the three germ layers formed in early embryonic development; this layer gives rise to tissues including the skin and the nervous system.

Mesoderm
One of the three embryonic germ layers; the mesoderm gives rise to connective tissue, the heart and blood, among other tissue types.

Table 1 | **Protein signalling molecules used to manipulate cell fate**

Family name (number of members in mammals)	Family members used in directed differentiation		Sample applications	Sample refs
TGFβ superfamily (33)	Activators	Activin A	Induction of endoderm	87
			Induction of mesoderm	16,17
		BMP4	Induction of endoderm	29
		TGFβ3	Dopaminergic neuron differentiation	53
		TGFβ1	Retinal pigment epithelium differentiation	84
	Inhibitors	LeftyA	Ectoderm specification	19,28
		Cerberus	Ectoderm specification	28
		Follistatin	Mesoderm specification	16
		Noggin	Anterior neural induction	18
FGF family (23)	Activators	bFGF	Retinal determination; otic induction	18,19
		FGF2	Hepatocyte differentiation	29,56
		FGF8	Dopaminergic neuron differentiation	27
		FGF10	Hepatocyte differentiation; otic induction	19,29
WNT family (19)	Activators	WNT3A	Induction of endoderm	20,31
			Induction of mesoderm	16
	Inhibitors	DKK1, Frizzled 8	Induction of ectoderm	18,19
Hedgehog family (3)	Activators	SHH	Induction of motor neurons	24
			Induction of dopamine neurons	53

bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; DKK1, Dickkopf-related protein 1; FGF, fibroblast growth factor; SHH, sonic hedgehog; TGF, transforming growth factor.

the empirical optimization of growth-factor treatments is extremely laborious. For example, D'Amour *et al.*[20] tested many members of the TGFβ family, the WNT family and the FGF family, as well as other growth factors, to define ideal conditions for the differentiation of pancreatic cell types from human ESCs; each protein was applied at different stages of differentiation, for various durations, at several concentrations and in different types of cell culture media. In addition to their use in directing the differentiation of pluripotent cells, growth and trophic factors can be used to support the cells on completion of differentiation.

In spite of their successful application to many stages of directed-differentiation protocols, the long-term utility of growth factors in these protocols is, in our opinion, open to question. Recombinant factors are often produced in engineered bacterial or mammalian cells, traces of which may contaminate the final preparation. Furthermore, the extremely high cost of recombinant growth factors may limit their use in larger-scale differentiation procedures. Therefore, although recombinant growth factors may be ideal for research purposes, other methods may, ultimately, be more useful for producing large quantities of cells suitable for therapy.

Small molecules. Chemical compounds offer, in our opinion, an attractive alternative to the protein factors described above. In comparison to protein factors, small molecules are less expensive, have less lot-to-lot variability, are non-immunogenic and are more stable. For these reasons, cells differentiated using small molecules might be more readily translated to therapeutic transplantation than those treated with recombinant proteins[21,22]. As a result, there has been intense interest in the idea of replacing biological factors with chemical ones in differentiation protocols. Three main approaches can be considered to achieve this goal.

First, many recent studies have demonstrated the feasibility of directly substituting a small molecule for a protein factor to activate or suppress a given developmental signalling pathway (TABLE 2). Small-molecule agonists and antagonists of the Hedgehog pathway have proved very effective, both for motor neuron differentiation[23,24] and possible cancer treatments[25]. Similarly, studies of the TGFβ signalling pathway have identified SB-431542, a small molecule that antagonizes the Nodal receptors ALK4, ALK5 and ALK7 (REF. 26) and is now commonly used to block TGFβ signalling. SB-431542 can substitute for protein antagonists of TGFβ in the differentiation of neurons and hepatocytes from human ESCs[27–29]. Another antagonist of TGFβ signalling, SIS3 (specific inhibitor of SMAD3)[30], has also been used to facilitate the production of anterior ectoderm from mouse iPSCs[19].

Small molecules can also be used to inhibit signalling through a pathway for which an endogenous inhibitor is not known. This approach can be used either to direct differentiation, or as a tool to verify the importance of a particular signalling pathway in directing a given cell-fate decision. For example, no protein antagonist of the Hedgehog signalling pathway is known; instead, KAAD-cyclopamine has been used to diminish

Endoderm
One of the three embryonic germ layers; this layer produces tissues such as the gut, liver, pancreas and lungs.

Hedgehog signalling to allow the formation of gut tube endoderm from human ESCs[20,31]. Similarly, no protein antagonists of FGF signalling are known[13]. Therefore, the small molecule SU5402 was used as a chemical tool to verify the importance of FGF signalling in mouse otic lineage induction[19].

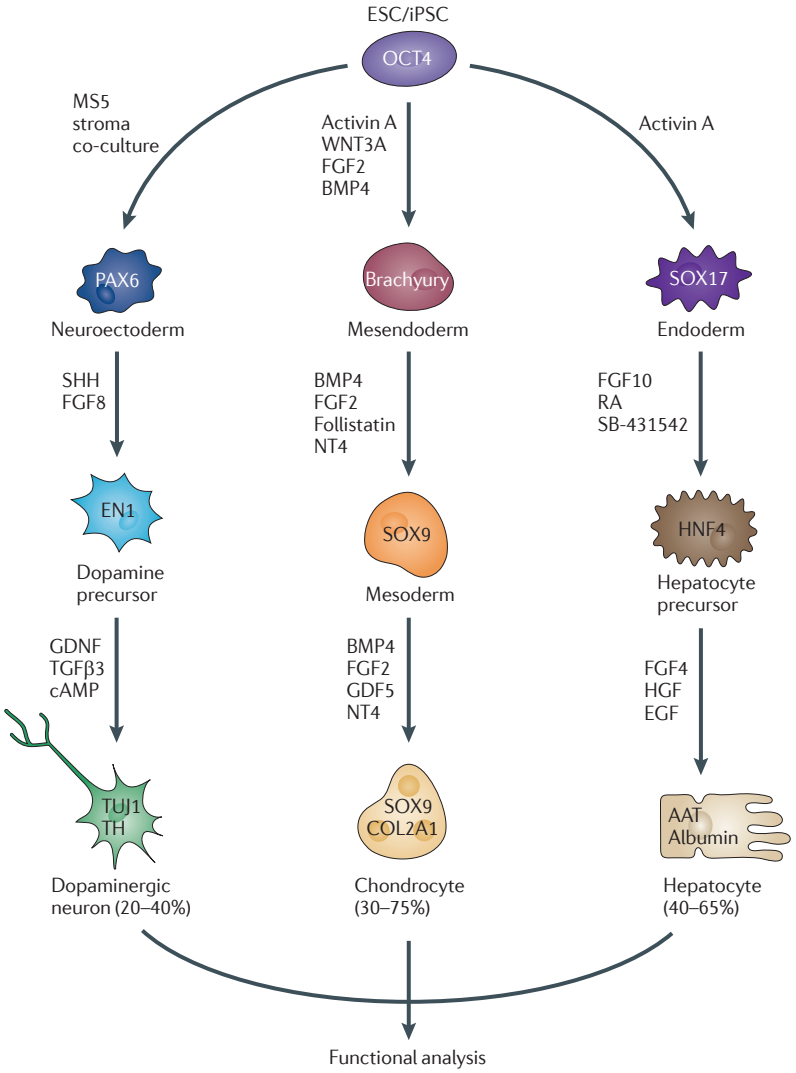


Figure 2 | **Directed differentiation.** This figure depicts current strategies by which pluripotent cells (embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), top) can be converted into any number of cell fates (bottom) by pushing the cell through a series of intermediate stages similar to those that occur during *in vivo* development[16,29,53]. At each stage, growth factors and small molecules (listed next to each arrow) can be used to push the differentiation of the cells towards the desired fate. At each stage, a representative marker used to identify cells is noted, although generally multiple markers are used to analyse the desired cells. Below the final stage, the approximate percentage of cells that achieve the desired fate, based on the analysis of different markers, is indicated. AAT, α1-antitrypsin; BMP4, bone morphogenetic protein 4; cAMP, cyclic AMP; COL2A1, collagen type II αI; EGF, epidermal growth factor; EN1, Engrailed homeobox 1; FGF, fibroblast growth factor; GDF5, growth differentiation factor 5; GDNF, glial-cell-derived neurotrophic factor; HGF, hepatocyte growth factor; HNF4, hepatocyte nuclear factor 4; NT4, neurotrophin 4; OCT4, octamer binding transcription factor 4 (also known as POU5F1); PAX6, paired box 6; RA, retinoic acid; SB-431542, TGFβ signalling inhibitor; SHH, sonic hedgehog; SOX, SRY (sex determining region Y)-box; TGFβ3, transforming growth factor-β3; TH, tyrosine hydroxylase; TUJ1, neuron specific class III β-tubulin.

Second, in cases in which the target biological pathway is not known, or molecules targeting the desired pathway have not been identified, chemical screening approaches can be used to identify, in a relatively unbiased manner, molecules that can produce the desired effect. The availability of high-content imaging systems has facilitated high-throughput screens based on marker gene expression (reviewed in REF. 21). However, so far, a limitation of the chemical screening approach has been the extreme difficulty in determining the mechanism of action of the identified compounds. So, although compounds found through chemical screening are extremely useful tools for controlling differentiation, their ability to provide new information about the biological mechanisms that control cell fate has been limited.

Chemical screening has been extensively applied to the search for molecules that can induce endoderm from ESCs in the hope of replacing the protein factor activin A. In one screen, 20,000 compounds were tested for their ability to enhance endoderm formation in mouse ESCs[32]. This screen, which was conducted in the presence of low levels of activin A, resulted in the identification of stauprimide, which substantially increased the number of endoderm cells in the cultures. However, this effect was completely dependent on the presence of activin A, and the compound showed no propensity to direct endoderm formation on its own. Follow-up experiments demonstrated that stauprimide acted to sensitize ESCs to a variety of differentiation signals[32]. A much smaller-scale study identified a compound (LY 294,002) that also enhances the production of endoderm when used in combination with activin A[33]. Although these compounds will be of use in directed-differentiation protocols, they fall short of the goal of completely replacing activin A.

In a third study[14] aimed at identifying small-molecule inducers of endoderm, the screen was conducted without activin A in the culture medium, ensuring that only molecules that could induce endoderm fate in the absence of activin A would be identified. Two hits, termed IDE-1 and IDE-2 (for 'inducer of endoderm'), emerged from this screen. Both could induce endoderm more robustly than activin A treatment. Interestingly, these compounds activate the TGFβ signalling pathway through an as yet unknown mechanism[14].

Other screening experiments have aimed to identify molecules that can push cells to a more advanced stage of differentiation. Surprisingly, in a screen seeking to find small-molecule inducers of cardiomyocytes from ESCs[34], ascorbic acid (vitamin C) was identified as the most efficacious compound. Its mechanism of action, although independent of its antioxidant effects, remains unclear[34]. A study to find molecules that could induce the formation of pancreatic progenitors identified indolactam V as a top candidate[31]. Alterations in cell fate produced by this molecule seemed to result from the activation of protein kinase C (PKC) signalling, although the most relevant PKC isoform was not identified[31].

Finally, endogenous small molecules that have roles in embryonic development may also be used *in vitro* to control differentiation. Retinoic acid, a

Table 2 | **Small molecules used to direct differentiation**

Molecule name	Function	Effect/use	Refs
Ascorbic acid	Not known	Dopamine and motor neuron differentiation	27
		Cardiac differentiation	34
Nicotinamide	Not known	Retinal pigment epithelium differentiation	84
Retinoic acid	Endogenous small molecule	Neuronal protocols	24
		Retinal protocols	35
Taurine	Endogenous small molecule	Retinal differentiation	35
PD173074	FGF inhibitor	Blocks endogenous caudalizing signals in motor neuron differentiation	86
SU5402	FGF inhibitor	Blocks otic induction	19
Hh.Agf.3	Hedgehog agonist	Induces motor neurons	24
C61414	Hedgehog antagonist	Blocks motor neuron induction	86
KAAD–cyclopamine	Hedgehog antagonist	Induces pancreatic cells from endoderm	20,31
LY294002	Phosphoinositide 3-kinase inhibitor	Enhances activin A signalling to generate endoderm	29,33
Indolactam V	Protein kinase C inhibitor	Induces pancreatic progenitors from endoderm	31
ALK inhibitor (SB-431542)	TGFβ signalling inhibitor (inhibitor of activin/Nodal signalling)	Neuron and hepatocyte differentiation	27–29
SIS3	TGFβ signalling inhibitor (inhibits SMAD3)	Otic induction	19

ALK, activin receptor-like kinase; FGF, fibroblast growth factor; KAAD, 3-keto-N-(aminoethyl-aminocaproyl-dihydrocinnamoyl); SIS3, specific inhibitor of SMAD3; TGF, transforming growth factor.

form of vitamin A, is an endogenous morphogen that is important in the patterning of the central nervous system (reviewed in REF. 19). It has been used successfully in a number of *in vitro* differentiation protocols, particularly those to generate neuronal or retinal cells from ESCs[24,35]. In these contexts, it seems to work synergistically with protein signalling molecules such as sonic hedgehog, FGFs and BMPs to provide patterning information to the differentiating neuronal precursors — for example by causing differentiating rostral-like cells to develop a more caudal character[24]. Similarly, the naturally occurring small molecule taurine has been used to direct the differentiation of retinal cells[35].

Spontaneous differentiation. Although the growth-factor- and small-molecule-based approaches to directing differentiation are, in our opinion, the most likely to produce uniform populations of differentiated cells, our current ability to control differentiation is extremely limited. Thus, in some instances, the only way to produce cells of a desired type from pluripotent cells is through the process of spontaneous differentiation. ESCs and iPSCs of human or mouse origin can give rise to diverse cell types *in vitro* through the spontaneous differentiation of floating clumps of cells, termed embryoid bodies. A disadvantage is that differentiation can vary depending on the starting size of the embryoid bodies[36]. Although stringent control of embryoid-body size[37] or allowing spontaneous differentiation to occur in an adherent monolayer[38] may reduce this variability to some degree, in all cases the desired cell type will be in a mixed population of many other cell types. In spite of these limitations, spontaneous differentiation in embryoid bodies can be a useful starting point in a directed-differentiation protocol, especially for neuronal cells[24,39] and cardiomyocytes[17,40]. In these cases, growth factors and small molecules can be applied after a period of spontaneous differentiation to increase the abundance of the desired cell type in the differentiating population.

Co-culture systems. Our ability to use recombinant growth factors to control differentiation is limited by our knowledge of which factors or combinations thereof will induce the desired changes in cell state. Co-culture systems, in which differentiating cells are plated on a layer of supporting cells, can provide the differentiating cells with the appropriate environment to guide their differentiation. This environment may consist of cell–cell contacts, the secretion of a complex mixture of factors, or both; detailed mechanisms by which supporting cells direct the differentiation of pluripotent cells remain largely undefined. Nonetheless, co-culture approaches remain useful for generating cells for research purposes when such cells cannot yet be produced using chemically defined conditions.

Supporting cells for co-culture can be derived from the physical location in the embryo in which the desired cell type emerges, so recapitulating that niche *in vitro*. For instance, to guide neuroectodermal cells to become hair cells, a co-culture system was used in which differentiating mouse iPSCs were grown atop cells isolated from embryonic chicken utricle (a region of the inner ear)[19]. Similarly, reaggregating human

Spontaneous differentiation
The process by which pluripotent stem cells take on a mixture of cell fates *in vitro* on transfer from media containing factors that maintain pluripotency to media lacking such factors.

Embryoid bodies
Clusters of pluripotent stem cells, usually grown in suspension culture, that are undergoing spontaneous differentiation.

ESC-derived retinal precursors with embryonic mouse retinal cells led to an increase in the number of ESC-derived cells expressing the photoreceptor marker rhodopsin[41].

Co-culture using primary cells requires frequent embryo dissections, rendering scale-up extremely difficult. Immortalized cell lines are an alternative; such cell lines can be derived from a region of the embryo that is known to be important in supporting the differentiation of a particular cell type. Heart development, for example, depends on interactions between cardiac progenitors and visceral endoderm[42]. On the basis of this knowledge, it was found that the visceral endoderm-like cell line END-2 (REF. 43) could support the differentiation of cardiomyocytes from ESCs[44].

Supporting cell lines can also be derived from an entirely different developmental stage or location from the desired cell type. Therefore, their ability to support a particular differentiation step must be viewed as a useful coincidence, rather than an aspect of their normal biological function. The stromal cell line PA6 (REF. 45), for instance, is derived from skull bone marrow, yet it is a potent inducer of differentiation of many neuronal types, a property known as stromal-cell-derived inducing activity (SDIA)[24,46].

Although co-culture systems may be convenient in generating certain cell types for research purposes, the differentiated cells cannot be transplanted into humans owing to the risk of contamination with animal pathogens or potentially tumorigenic co-culture cells[47]. Therefore, for therapeutic applications, it will ultimately be necessary to replace the support cells. However, identifying factors with which to replace them is far from simple. For instance, after 10 years of study, most work favours the idea that SDIA is the result of secreted factors, although which factors are the most important is still a matter of debate (see, for example, REFS 48–50).

Current limitations and challenges. Each directed-differentiation protocol is typically developed using one pluripotent stem cell line (either ESCs or iPSCs derived from either humans or mice) as a substrate. Different human ESC and iPSC lines may have substantially different propensities to differentiate into different lineages[51,52]. Thus, even if a particular protocol is quite efficient at directing the differentiation of the cell line for which it was devised, it might be less efficient for others[31]. Further complications can arise when a protocol developed in mouse cells is adapted for use with human cells. Although cases exist in which discoveries have been translated from mouse cells to human cells and vice versa[14,31], alterations in protocol parameters may be required to achieve maximal differentiation efficiency.

Even in the best-case scenario, when an optimized protocol is used with a receptive cell line, the efficiency with which cells of the desired fate are produced is quite low, often 30% or less. Induction of the desired germ layer is typically the most efficient step of a directed-differentiation protocol but, even in this case, at most 80% of cells typically achieve the desired identity (for example, REFS 16,31,53). The inability to achieve 100% efficiency even at the first stage of a differentiation protocol may be due, in part, to the finding that not all pluripotent stem cells in a population are equivalent in their propensity to differentiate[54,55]. We hypothesize that the extremely low efficiency of subsequent steps in a directed-differentiation protocol might be attributable to the emergence of distinct sub-populations of differentiating cells that are endowed with varying propensities to adopt particular cell fates. However, this idea has yet to be rigorously tested through the analysis of single cells. It is also possible that the cellular niche, including the extracellular matrix and three-dimensional configuration of cells in the culture, are crucial parameters that must also be optimized to achieve efficient differentiation.

Another important limitation of current technologies is that directed-differentiation protocols produce, almost without exception, immature cells with embryonic or early postnatal phenotypes, rather than truly adult cells[16,19,20,29,56]. We suggest that this may be a reflection of the fact that whereas most directed-differentiation protocols involve culturing cells for several weeks, the development of fully mature cells in either mice or humans takes much longer. Alternatively, we note the possibility that the relevant cell type may require signals, support or an appropriate three-dimensional setting, as provided by an *in vivo* niche, to become fully mature. Indeed, it has been found that the implantation into an adult mouse of immature cells produced by directed differentiation of human cells can support their continued maturation over a period of months[15]. Studies such as this suggest that perhaps, even if adult cell fates cannot be achieved *in vitro*, the products of directed differentiation may still be valuable for therapeutic transplantation, provided that concerns about the tumorigenic potential of such cells can be adequately addressed. However, for *in vitro* disease research and drug screening, it will be necessary to find ways to push cells towards more mature phenotypes. Screening and protocol-optimization approaches similar to those undertaken to identify ideal conditions for each of the other steps in a directed-differentiation protocol seem likely to identify the culture conditions needed to push cells to a mature state.

Reprogramming

The feasibility of directly converting one cell type into another was first demonstrated in 1987, when it was shown that overexpression of MyoD was sufficient to convert mouse fibroblasts into myoblasts[57]. More recently, the finding that adult somatic cells can be reprogrammed to a pluripotent state demonstrated that drastic alterations in cell fate could be achieved with a combination of factors when no single factor would suffice[58]. This has opened the door to searches for factors that can drive the transdifferentiation of readily available cells, such as fibroblasts, to therapeutically desirable cells, such as neurons[6] and cardiomyocytes[7].

Multiplicity of infection
The ratio of viral particles present in a transduction experiment divided by the number of target cells present.

General reprogramming strategy. On the basis of the approach taken by Shinya Yamanaka and colleagues to generate iPSCs[58], the first step in any reprogramming study is the identification of factors to be tested for their ability to contribute to the desired reprogramming event (FIG. 3). The concept that transcription factors are the key mediators of cellular identity has gained widespread acceptance, so lists of transcription factors (and, more rarely, chromatin-remodelling factors[7]) are generally compiled. Transcription factors that are highly expressed in the target cell type, as well as those known to play a part in the development of that cell type, are selected. Overexpression of particular receptors to sensitize the starting cells to certain signalling pathways[59] or the ablation of factors important for maintaining the identity of the starting cell[60] may also be considered.

Once the list of reprogramming factors has been assembled, viral expression constructs designed to overexpress each factor must be produced. The factors are often expressed under the control of a ubiquitous promoter to ensure their robust expression in a variety of cell types. However, because temporal control of the expression of the reprogramming factors is highly desirable, two strategies have been used to establish inducible reprogramming-factor action. First, the reprogramming factors can be placed under the control of a doxycycline-inducible promoter[6,7]. Second, a fusion protein consisting of the reprogramming factor and the hormone-binding domain of the oestrogen receptor (ER) can be generated[61]. In untreated cells, ER traps the reprogramming factor in the cytosol; after β-oestradiol treatment, the fusion protein is translocated to the nucleus so that the reprogramming factor can act[61]. Importantly, inducible systems enable assessment of the stability of the reprogrammed cell's identity after reprogramming-factor withdrawal.

Selection of a starting cell population is also a crucial decision, because it has long been known that not all cell types are equally amenable to a given reprogramming event[62]. One important consideration is the developmental relationship between the two cell types. It is often thought that reprogramming may be easier if the starting cell type shares a common developmental history with the desired cell type. For instance, many different interconversions of cell types in the haematopoietic lineage, which arises entirely from haematopoietic stem cells, have been reported in mice[59–61,63]. Likewise, mouse pancreatic acinar cells can be converted into a related cell type, insulin-producing β-cells, with the use of three transcription factors[64]. However, a growing number of examples are emerging in which a starting cell has been successfully converted to a target cell type that arises from a different germ layer. Examples of this include the production of cardiomyocytes from postnatal mouse cardiac or dermal fibroblasts[7], and the generation of blood-cell progenitors from human dermal fibroblasts[8]. These studies suggest that a shared developmental history between the starting cell and the desired cell type is not required in all cases.

After the candidate reprogramming factors and the starting cell type have been chosen, the viruses encoding the reprogramming factors are typically applied to the starting cells as a 'pool'. Expression of GFP from a reporter transgene[6,7] or immunodetection of the expression of an endogenous marker gene[8,63,64] is then used to detect reprogramming events. The initial pool can be refined by omitting individual viruses or by testing smaller 'sub-pools' of viruses. This allows the initial list to be whittled down to a small core group of reprogramming factors. In rare cases, a single factor may be sufficient for reprogramming[57,61,63,65,66]. Reducing the number of factors in the reprogramming pool often increases the efficiency of the reprogramming procedure because it results in a higher multiplicity of infection for each of the active reprogramming factors[7].

Reprogramming: key challenges. Most currently available reprogramming strategies have been developed using mouse cells. One important reason for this is the

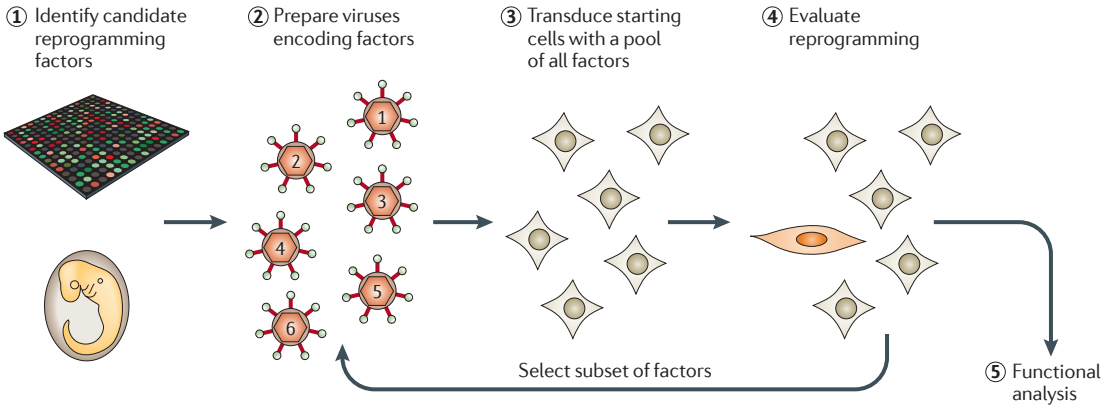


Figure 3 | **Reprogramming.** A schematic for creating a reprogramming protocol. Candidate reprogramming factors are identified on the basis of their expression patterns (step **1**) as determined by analysis of embryos or through the use of microarrays. Viruses are generated that encode these factors (step **2**) and are used to transduce a starting population of cells (step **3**). Transduced cells are evaluated to identify reprogramming events (step **4**). On the basis of this evaluation, a subset of factors may be selected for retesting in the reprogramming assay (arrow leading back) Finally, reprogrammed cells must be evaluated to determine their functional similarity to primary cells of the same type (step **5**).

availability of transgenic mouse lines in which GFP is expressed under the control of a lineage-specific promoter. Such lines facilitate the screening of reprogramming factors because reprogrammed cells can be identified quickly by the expression of GFP. Furthermore, lists of candidate reprogramming factors are generally compiled on the basis of studies of gene expression and development in mice. These factors may therefore be either inappropriate or insufficient for the reprogramming of human cells. Although the cocktail of factors discovered for the reprogramming of somatic cells to iPSCs was successfully translated to human cells[67–69], it remains to be seen whether this will be true for any of the other reprogramming factors described above.

Even assuming that factors that can reprogramme human cells into therapeutically relevant cell types can be identified, cells reprogrammed using viruses are unlikely to gain acceptance for transplantation into human patients, because the viruses permanently integrate into the host genome. In light of this concern, several reprogramming technologies that do not rely on viral integration have been developed for the production of iPSCs, and these may be applicable to reprogramming more generally; approaches include the use of non-integrating viruses[70–72], the treatment of cells with cell-penetrant reprogramming factor proteins[73], transposon-based systems[74] and the delivery of reprogramming factors on plasmids[75–77]. Although these strategies eliminate the threat of random viral integration into the host cell genome, they are generally more technically demanding and less efficient than viral transduction and, as a result, have not been widely adopted. Recently, repeated transfection of modified mRNA encoding the reprogramming factors has been shown to be efficient for generating iPSCs[78]. This approach may hold promise for other reprogramming applications. Another technique that could be investigated further is the use of small molecules to either increase the efficiency of reprogramming or to replace individual reprogramming factors, as has been done for the generation of iPSCs[79–82]. A recent screen identified a small molecule that induces insulin expression in an immortalized pancreatic α-cell line, suggesting that the cells had been at least partly reprogrammed to a β-cell fate[83]. Further studies will be needed to address the utility of modified RNAs and small molecules for the reprogramming of adult primary cells.

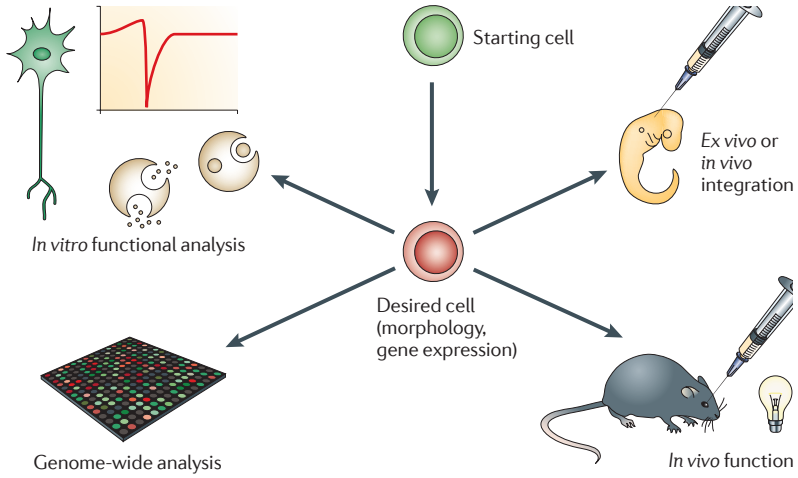
Box 1 | **Evaluation of directed-differentiation and reprogramming products**

***In vitro* analysis**

Generally, cells are first screened using simple assays such as immunofluorescent or reverse transcription PCR detection of marker gene expression to ensure that the desired genes are expressed and that genes typical of the starting cell type are not. The morphology of the cells may also be examined to ensure that they resemble the desired cell type (see the figure, centre). Assuming that these parameters are met, the cells may be tested *in vitro* (top left) to determine whether they have other properties of the desired cell type, such as electrical responses and secretion of particular factors, or behaviours such as phagocytosis. Genome-wide analysis (bottom left) may be used to compare the transcriptional profile and histone or DNA-modification profile of the created cells to primary cells of the same type, assuming that such cells can be isolated at sufficient quantity and purity.

***Ex vivo* and *in vivo* analysis**

If these *in vitro* assays show that the created cells closely resemble the desired primary cell type, the cells can be tested for their ability to integrate into the appropriate anatomical niche, either *in vivo* or *ex vivo*. For example, motor neurons generated from induced pluripotent stem cells can be injected into the spinal cord of the chick embryo and tested for their ability to correctly extend processes to the limbs (top right; see also REF. 86). In some cases, preclinical models that are defective in a particular cell type are available, allowing the functionality of cells created *in vitro* to be tested directly. Rats lacking functional retinal pigment epithelium (RPE) can be transplanted with RPE generated from human embryonic stem cells, restoring the ability of the eye to respond to light[84] (bottom right).



Functional analysis of differentiated cells

An important concern for directed-differentiation protocols and reprogramming-based strategies is the evaluation of the cells produced. The expression of marker genes is used as the first-pass analysis to determine whether a given manipulation might have produced the desired change in cell fate (BOX 1). Marker genes are typically selected on the basis of studies of developmental biology (usually conducted in the mice) in which a particular gene was shown to be expressed only in a given cell type in the embryo. Often, combinations of marker genes are used to reduce the risk of false read-out from any one gene. Manipulated cells may also be checked to ensure that they do not express markers of the starting cell type, or markers of cell types related to but distinct from that desired. Analysis of cellular morphology can provide further information about the successful generation of a particular cell type. When possible, it can also be informative to compare the transcription profile, DNA methylation and histone modifications of the differentiated cells with that of their *in vivo* counterparts (see REF. 7 for an example).

Functional tests must be used to determine whether the cells exhibit similar physiological behaviours to those of same type *in vivo*. For instance, hepatocytes secrete albumin, take up low-denisty lipoprotein and store glycogen, among other functions, and these properties can all be assayed to confirm the identity of ESC-derived cells expressing hepatocyte markers[29,56]. Electrophysiological measurements can be used to query the functionality of cells such as neurons, cardiomyocytes, retinal cells and hair cells[18,19,44,53]. Cells can also be tested for their ability

to integrate into the appropriate anatomical setting either *ex vivo* or *in vivo* (examples include REFS 39,46). The most stringent functional test for any cell produced *in vitro* is determining whether the cell can functionally replace the same cell type *in vivo*. In practice, it is rare to find animal models entirely lacking a particular cell type that can be used in such testing, although a few instances have been reported[15,84,85]. Thus, establishing sufficiently stringent tests for cellular identity and function, both *in vitro* and *in vivo*, remains a significant challenge.

Future directions

In this Review, we have described the impressive progress that has been made in developing strategies to control cell fate. The future development of these methods has great potential to generate cells for study and transplantation therapy. At the moment, it remains unclear whether one strategy will predominate as a method for controlling cell fate, or whether a combination of the methods will be more successful than either a directed-differentiation or reprogramming strategy alone.

We see several obstacles that must be overcome before the cells generated can be used widely in the clinic. First, current protocols have all been developed on a scale suited to the research laboratory, and so significant work must be invested in scaling up available protocols to produce sufficient cells to treat a human patient. Moreover, many of the current protocols have been developed in mouse model systems; they need to be translated to the human system before their full potential for research and therapy can be realized. In addition, most current protocols, regardless of species or strategy, are extremely inefficient at producing the desired cells. More efficient protocols need to be developed, in concert with improved methods for separating the desired cells from other cells in a preparation. As noted above, strategies to force cells with embryonic phenotypes into a more mature state must also be defined. Finally, manipulations that render the cells unsuitable for use in humans, such as viral integration and co-culture with animal cells, must be eliminated.

Although these issues remain unsolved for the majority of protocols, it is encouraging to note that two Phase I clinical trials of human ESC derivatives are currently under way (by Geron (GRNOPC1) and Advanced Cell Technology). We anticipate that the number of such trials will grow, and that cells generated from protocols similar to those described here will ultimately contribute significantly to the study and treatment of human disease.

1. Lengner, C. J. iPS cell technology in regenerative medicine. *Ann. N. Y. Acad. Sci.* **1192**, 38–44 (2010).
2. Laustriat, D., Gide, J. & Peschanski, M. Human pluripotent stem cells in drug discovery and predictive toxicology. *Biochem. Soc. Trans.* **38**, 1051–1057 (2010).
3. Park, I. H. *et al.* Disease-specific induced pluripotent stem cells. *Cell* **134**, 877–886 (2008).
4. Kiskinis, E. & Eggan, K. Progress toward the clinical application of patient-specific pluripotent stem cells. *J. Clin. Invest.* **120**, 51–59 (2010).
5. Zhou, Q. & Melton, D. A. Extreme makeover: converting one cell into another. *Cell Stem Cell* **3**, 382–388 (2008).
6. Vierbuchen, T. *et al.* Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035–1041 (2010).
This paper was the first to demonstrate the feasibility of producing neurons, a cell type that is highly desirable for both study and therapy, using a reprogramming strategy.
7. Ieda, M. *et al.* Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* **142**, 375–386 (2010).
This work demonstrates that fibroblasts, which are a derivative of the ectoderm, can be reprogrammed into a mesodermal derivative, cardiomyocytes.
8. Szabo, E. *et al.* Direct conversion of human fibroblasts to multilineage blood progenitors. *Nature* **468**, 521–526 (2010).
9. Manohar, R. & Lagasse, E. Transdetermination: a new trend in cellular reprogramming. *Mol. Ther.* **17**, 936–938 (2009).
10. Wagers, A. J. & Weissman, I. L. Plasticity of adult stem cells. *Cell* **116**, 639–648 (2004).
11. Moustakas, A. & Heldin, C. H. The regulation of TGFβ signal transduction. *Development* **136**, 3699–3714 (2009).
12. Rao, T. P. & Kuhl, M. An updated overview on Wnt signaling pathways: a prelude for more. *Circ. Res.* **106**, 1798–1806 (2010).
13. Turner, N. & Grose, R. Fibroblast growth factor signalling: from development to cancer. *Nature Rev. Cancer* **10**, 116–129 (2010).
14. Borowiak, M. *et al.* Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. *Cell Stem Cell* **4**, 348–358 (2009).
15. Kroon, E. *et al.* Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. *Nature Biotech.* **26**, 443–452 (2008).
16. Oldershaw, R. A. *et al.* Directed differentiation of human embryonic stem cells toward chondrocytes. *Nature Biotech.* **28**, 1187–1194 (2010).
17. Yang, L. *et al.* Human cardiovascular progenitor cells develop from a KDR$^+$ embryonic-stem-cell-derived population. *Nature* **453**, 524–528 (2008).
18. Lamba, D. A., Karl, M. O., Ware, C. B. & Reh, T. A. Efficient generation of retinal progenitor cells from human embryonic stem cells. *Proc. Natl Acad. Sci. USA* **103**, 12769–12774 (2006).
19. Oshima, K. *et al.* Mechanosensitive hair cell-like cells from embryonic and induced pluripotent stem cells. *Cell* **141**, 704–716 (2010).
This elegant study used various methods, including growth factors, small molecules and co-culture, to produce an extremely scarce cell type, the hair cell of the ear, by directed differentiation.
20. D'Amour, K. A. *et al.* Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nature Biotech.* **24**, 1392–1401 (2006).
21. Ding, S. & Schultz, P. G. A role for chemistry in stem cell biology. *Nature Biotech.* **22**, 833–840 (2004).
22. Rubin, L. L. Stem cells and drug discovery: the beginning of a new era? *Cell* **132**, 549–552 (2008).
23. Frank-Kamenetsky, M. *et al.* Small-molecule modulators of Hedgehog signaling: identification and characterization of Smoothened agonists and antagonists. *J. Biol.* **1**, 10 (2002).
This work identified small-molecule modulators of the Hedgehog pathway, a developmental signalling network that is important to the specification of many tissues. This demonstrates the ability of small molecules to significantly influence cell-fate decisions through a known mechanism.
24. Wichterle, H., Lieberam, I., Porter, J. A. & Jessell, T. M. Directed differentiation of embryonic stem cells into motor neurons. *Cell* **110**, 385–397 (2002).
25. Rubin, L. L. & de Sauvage, F. J. Targeting the Hedgehog pathway in cancer. *Nature Rev. Drug Discov.* **5**, 1026–1033 (2006).
26. Laping, N. J. *et al.* Inhibition of transforming growth factor (TGF)-β1-induced extracellular matrix with a novel inhibitor of the TGF-β type I receptor kinase activity: SB-431542. *Mol. Pharmacol.* **62**, 58–64 (2002).
27. Chambers, S. M. *et al.* Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nature Biotech.* **27**, 275–280 (2009).
28. Smith, J. R. *et al.* Inhibition of Activin/Nodal signaling promotes specification of human embryonic stem cells into neuroectoderm. *Dev. Biol.* **313**, 107–117 (2008).
29. Touboul, T. *et al.* Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. *Hepatology* **51**, 1754–1765 (2010).
30. Jinnin, M., Ihn, H. & Tamaki, K. Characterization of SIS3, a novel specific inhibitor of Smad3, and its effect on transforming growth factor-β1-induced extracellular matrix expression. *Mol. Pharmacol.* **69**, 597–607 (2006).
31. Chen, S. *et al.* A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nature Chem. Biol.* **5**, 258–265 (2009).
This study demonstrates the efficacy of chemical screening for the identification of compounds that can affect cell fate — in this case, the conversion of endoderm cells into pancreatic progenitors.
32. Zhu, S. *et al.* A small molecule primes embryonic stem cells for differentiation. *Cell Stem Cell* **4**, 416–426 (2009).
33. McLean, A. B. *et al.* Activin A efficiently specifies definitive endoderm from human embryonic stem cells only when phosphatidylinositol 3-kinase signaling is suppressed. *Stem Cells* **25**, 29–38 (2007).
34. Takahashi, T. *et al.* Ascorbic acid enhances differentiation of embryonic stem cells into cardiac myocytes. *Circulation* **107**, 1912–1916 (2003).
35. Osakada, F. *et al.* In vitro differentiation of retinal cells from human pluripotent stem cells by small-molecule induction. *J. Cell Sci.* **122**, 3169–3179 (2009).
36. Dhara, S. K. & Stice, S. L. Neural differentiation of human embryonic stem cells. *J. Cell. Biochem.* **105**, 633–640 (2008).
37. Ng, E. S., Davis, R. P., Azzola, L., Stanley, E. G. & Elefanty, A. G. Forced aggregation of defined numbers of human embryonic stem cells into embryoid bodies fosters robust, reproducible hematopoietic differentiation. *Blood* **106**, 1601–1603 (2005).
38. Ying, Q. L., Stavridis, M., Griffiths, D., Li, M. & Smith, A. Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. *Nature Biotech.* **21**, 183–186 (2003).
39. Di Giorgio, F. P., Boulting, G. L., Bobrowicz, S. & Eggan, K. C. Human embryonic stem cell-derived motor neurons are sensitive to the toxic effect of glial cells carrying an ALS-causing mutation. *Cell Stem Cell* **3**, 637–648 (2008).

40. Kehat, I. *et al.* Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J. Clin. Invest.* **108**, 407–414 (2001).
41. Ikeda, H. *et al.* Generation of Rx$^+$/Pax6$^+$ neural retinal precursors from embryonic stem cells. *Proc. Natl Acad. Sci. USA* **102**, 11331–11336 (2005).
42. Arai, A., Yamamoto, K. & Toyama, J. Murine cardiac progenitor cells require visceral embryonic endoderm and primitive streak for terminal differentiation. *Dev. Dyn.* **210**, 344–353 (1997).
43. Mummery, C. L. *et al.* Visceral-endoderm-like cell lines induce differentiation of murine P19 embryonal carcinoma cells. *Differentiation* **46**, 51–60 (1991).
44. Mummery, C. *et al.* Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation* **107**, 2733–2740 (2003).
45. Kodama, H. *et al.* MC3T3-G2/PA6 preadipocytes support *in vitro* proliferation of hemopoietic stem cells through a mechanism different from that of interleukin 3. *J. Cell. Physiol.* **129**, 20–26 (1986).
46. Kawasaki, H. *et al.* Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron* **28**, 31–40 (2000).
47. Sun, N., Longaker, M. T. & Wu, J. C. Human iPS cell-based therapy: considerations before clinical applications. *Cell Cycle* **9**, 880–885 (2010).
48. Ishii, S. *et al.* Stromal cell-secreted factors promote the survival of embryonic stem cell-derived early neural stem/progenitor cells via the activation of MAPK and PI3K-Akt pathways. *J. Neurosci. Res.* **88**, 722–734 (2010).
49. Swistowska, A. M. *et al.* Stage-specific role for shh in dopaminergic differentiation of human embryonic stem cells induced by stromal cells. *Stem Cells Dev.* **19**, 71–82 (2010).
50. Vazin, T. *et al.* A novel combination of factors, termed *SPIE*, which promotes dopaminergic neuron differentiation from human embryonic stem cells. *PLoS ONE* **4**, e6606 (2009).
51. Kim, D. S. *et al.* Robust enhancement of neural differentiation from human ES and iPS cells regardless of their innate difference in differentiation propensity. *Stem Cell Rev.* **6**, 270–281 (2010).
52. Osafune, K. *et al.* Marked differences in differentiation propensity among human embryonic stem cell lines. *Nature Biotech.* **26**, 313–315 (2008).
53. Perrier, A. L. *et al.* Derivation of midbrain dopamine neurons from human embryonic stem cells. *Proc. Natl Acad. Sci. USA* **101**, 12543–12548 (2004).
54. Chambers, I. *et al.* Nanog safeguards pluripotency and mediates germline development. *Nature* **450**, 1230–1234 (2007).
55. Fischer, Y. *et al. NANOG* reporter cell lines generated by gene targeting in human embryonic stem cells. *PLoS ONE* **5**, e12533 (2010).
56. Rashid, S. T. *et al.* Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. *J. Clin. Invest.* **120**, 3127–3136 (2010).
57. Davis, R. L., Weintraub, H. & Lassar, A. B. Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51**, 987–1000 (1987).
58. Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676 (2006).
59. Kondo, M. *et al.* Cell-fate conversion of lymphoid-committed progenitors by instructive actions of cytokines. *Nature* **407**, 383–386 (2000).
60. Cobaleda, C., Jochum, W. & Busslinger, M. Conversion of mature B cells into T cells by dedifferentiation to uncommitted progenitors. *Nature* **449**, 473–477 (2007).
61. Bussmann, L. H. *et al.* A robust and highly efficient immune cell reprogramming system. *Cell Stem Cell* **5**, 554–566 (2009).
62. Schafer, B. W., Blakely, B. T., Darlington, G. J. & Blau, H. M. Effect of cell history on response to helix-loop-helix family of myogenic regulators. *Nature* **344**, 454–458 (1990).
63. Xie, H., Ye, M., Feng, R. & Graf, T. Stepwise reprogramming of B cells into macrophages. *Cell* **117**, 663–676 (2004).
64. Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J. & Melton, D. A. *In vivo* reprogramming of adult pancreatic exocrine cells to β-cells. *Nature* **455**, 627–632 (2008).
The authors of this paper succeeded in reprogramming pancreatic exocrine cells into insulin-producing β-cells *in vivo* through the expression of three reprogramming factors.
65. Collombat, P. *et al.* Embryonic endocrine pancreas and mature β cells acquire α and PP cell phenotypes upon Arx misexpression. *J. Clin. Invest.* **117**, 961–970 (2007).
66. Collombat, P. *et al.* The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into α and subsequently β cells. *Cell* **138**, 449–462 (2009).
67. Park, I. H. *et al.* Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* **451**, 141–146 (2008).
68. Takahashi, K. *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872 (2007).
69. Yu, J. *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917–1920 (2007).
70. Nishimura, K. *et al.* Development of defective and persistent sendai virus vector: a unique gene delivery/expression system ideal for cell reprogramming. *J. Biol. Chem.* **286**, 4760–4771 (2010).
71. Stadtfeld, M., Nagaya, M., Utikal, J., Weir, G. & Hochedlinger, K. Induced pluripotent stem cells generated without viral integration. *Science* **322**, 945–949 (2008).
72. Zhou, W. & Freed, C. R. Adenoviral gene delivery can reprogram human fibroblasts to induced pluripotent stem cells. *Stem Cells* **27**, 2667–2674 (2009).
73. Kim, D. *et al.* Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* **4**, 472–476 (2009).
74. Woltjen, K. *et al. piggyBac* transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* **458**, 766–770 (2009).
75. Okita, K., Nakagawa, M., Hyenjong, H., Ichisaka, T. & Yamanaka, S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* **322**, 949–953 (2008).
76. Si-Tayeb, K. *et al.* Generation of human induced pluripotent stem cells by simple transient transfection of plasmid DNA encoding reprogramming factors. *BMC Dev. Biol.* **10**, 81 (2010).
77. Yu, J. *et al.* Human induced pluripotent stem cells free of vector and transgene sequences. *Science* **324**, 797–801 (2009).
78. Warren, L. *et al.* Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* **7**, 618–630 (2010).
This paper describes the transfection of modified mRNAs as a means of expressing reprogramming factors in a target cell without permanent genetic modification of the cell. The high efficiency of this method, combined with the potential to make mRNA encoding any reprogramming factor of interest, suggest that this method may gain widespread use for various reprogramming applications.
79. Huangfu, D. *et al.* Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nature Biotech.* **26**, 795–797 (2008).
80. Huangfu, D. *et al.* Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nature Biotech.* **26**, 1269–1275 (2008).
81. Lyssiotis, C. A. *et al.* Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of Klf4. *Proc. Natl Acad. Sci. USA* **106**, 8912–8917 (2009).
82. Shi, Y. *et al.* Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell* **3**, 568–574 (2008).
83. Fomina-Yadlin, D. *et al.* Small-molecule inducers of insulin expression in pancreatic α-cells. *Proc. Natl Acad. Sci. USA* **107**, 15099–15104 (2010).
84. Idelson, M. *et al.* Directed differentiation of human embryonic stem cells into functional retinal pigment epithelium cells. *Cell Stem Cell* **5**, 396–408 (2009).
85. Lamba, D. A., Gust, J. & Reh, T. A. Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in Crx-deficient mice. *Cell Stem Cell* **4**, 73–79 (2009).
86. Peljto, M., Dasen, J. S., Mazzoni, E. O., Jessell, T. M. & Wichterle, H. Functional diversity of ESC-derived motor neuron subtypes revealed through intraspinal transplantation. *Cell Stem Cell* **7**, 355–366 (2010).
87. D'Amour, K. A. *et al.* Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nature Biotech.* **23**, 1534–1541 (2005).

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The authors declare no competing financial interests.

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