REVIEWS

Forcing cells to change lineages

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The ability to produce stem cells by induced pluripotency (iPS reprogramming) has rekindled an interest in earlier studies showing that transcription factors can directly convert specialized cells from one lineage to another. Lineage reprogramming has become a powerful tool to study cell fate choice during differentiation, akin to inducing mutations for the discovery of gene functions. The lessons learnt provide a rubric for how cells may be manipulated for therapeutic purposes.

Seemingly at odds with the stability of the differentiated state in metazoa are cell fusion and nuclear transfer experiments, which have shown that the epigenomes of differentiated cells can be remarkably plastic. Experiments performed several decades ago showed that dormant gene expression programs can be dominantly awakened in differentiated cells by the fusion of different pairs of cell types[1]. Subsequently, lineage conversions could be effected simply through the introduction of defined transcription factors[2,3] (Fig. 1a, b). Parallel experiments, conducted in a number of different species, showed that transfer of nuclei from both embryonic and adult somatic cell types can lead to the formation of all three germ layers and even to the generation of entire new animals[4–7], unequivocally demonstrating that the identity of differentiated cells can be fully reversed. The latest and most dramatic development is the demonstration that somatic cells can be reprogrammed to a pluripotent state by the expression of a transcription factor cocktail, generating induced pluripotent stem (iPS, for nomenclature see Box 1) cells[8] (Fig. 1c).

The facility with which cell fates can be altered experimentally raises the question as to whether such interconversions occur physiologically or in the context of disease. Arguably, gastrulation provides a first example of transdetermination, where an invagination of the ectoderm produces mesoderm (reviewed in refs 9, 10). Transdetermination and transdifferentiation may also have a role in regeneration, metaplasia and cancer. For example, removing the eye lens of a newt leads to depigmentation of dorsal iris cells and their redifferentiation into

transparent lens cells consisting of specialized keratinocytes (reviewed in ref. 9). In another well-studied system of regeneration, limb regeneration in axolotls, it has long been assumed that the blastema that forms in response to injury contains de- and re-differentiating cells. However, recent work indicates that only dermis cells can 'transdifferentiate' into cartilage and tendons, whereas cartilage, muscle and neuronal precursors within the blastema do not change identity before generating a new limb[11]. Several types of metaplasia have been attributed to transdifferentiation[9], and epithelial mesenchymal transitions may be involved in the formation of metastatic breast cancers[10]. Here, as during normal epithelial mesenchymal transitions, activation of the transcription factors Snail, Slug and Twist are essential[9,10]. With the rapidly growing arsenal of lineage tracing tools it seems likely that many more physiological or pathogenic cell conversions will be discovered in the future.

Perhaps the best evidence that functionally differentiated cells can change fate during normal development comes from studies on the origin of blood. Fetal blood cells originate in the dorsal aorta after activation of Scl (also known as Tal1) and Runx1[12,13], two transcription factors essential for haematopoietic stem cell formation[14]. Most strikingly, time-lapse experiments recently showed that a small proportion of cells within cultured endothelial sheet colonies derived from embryonic stem (ES) cells undo their tight junctions, round up and begin to express erythroid and monocytic haematopoietic antigens[15] (Fig. 2). This process, which is unique to embryonic as
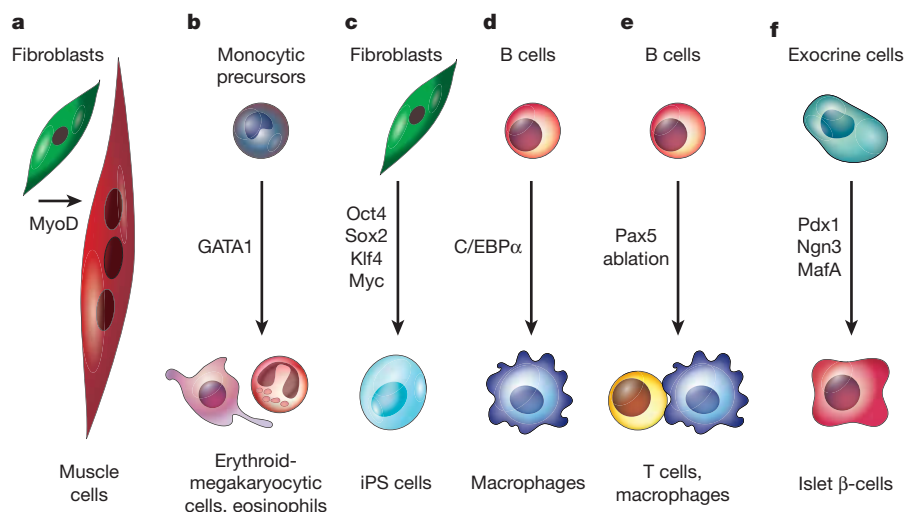

Figure 1 | Examples of transcription factor overexpression or ablation experiments that result in cell fate changes. For explanation of panels **a–f** see text.

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Box 1 | Glossary

Lineage Cells of the same developmental origin sharing a similar phenotype/function.
Cell differentiation Process by which cells become more specialized, acquiring new identities.
Cell determination Commitment to a lineage.
Commitment Stable activation of a gene expression program characteristic of a lineage.
Pluripotent Potential of a cell to generate all cell types except extra-embryonic tissue. Examples: embryonic stem cells and induced pluripotent stem (iPS) cells.
Multipotent Potential of a cell to form several lineages within a tissue. Example: haematopoietic stem cells.
Progenitor Cell with the capacity to differentiate and divide but with limited self-renewal potential.
iPS cell reprogramming Induced conversion of somatic cells into pluripotent stem cells.
Lineage reprogramming Conversion of cells from one lineage to another. Term covers both transdifferentiation and transdetermination.
Transdifferentiation Reprogramming of one specialized cell type into another, without reversion to pluripotent cells. Also called 'lineage switching' or 'lineage conversion'.
Transdetermination Reprogramming of a committed, but not yet fully differentiated, cell type into another.
Lineage priming Promiscuous expression in progenitors of transcriptional programs associated with different lineages.
Epigenome/epigenetics Changes in phenotype or gene expression caused by mechanisms other than changes in the underlying DNA.
Cell regeneration Replacement of cells lost by injury or attrition.
Plasticity Ability of a cell to convert into another cell type either spontaneously, by external cues or by gene perturbation experiments.
Metaplasia Replacement in a tissue of one differentiated cell type with another, generally caused by an abnormal chronic stimulus.

opposed to adult endothelium, is exacerbated by shear stress (mimicking blood flow) through production of nitric oxide, upregulation of Runx1, c-Myb and Klf2 (refs 16,17). Interestingly, c-Myb, like Runx1 and Scl, is a transcription factor that is also required for the formation of definitive blood cells[14]. The re-specification of endothelium into haematopoietic cells supports the notion that transdifferentiation may occur during normal development and alternates
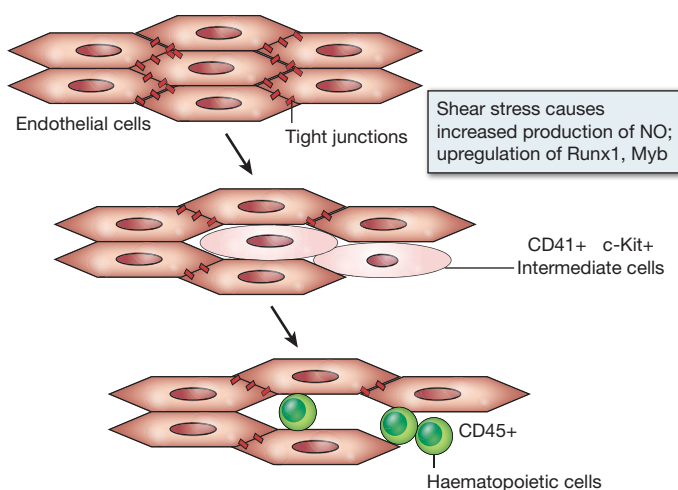

Figure 2 | Conversion of endothelial cells into haematopoietic cells. Schematic of time-lapse microscopy of endothelial colonies derived from ES cells showing that some cells round up and begin to express haematopoietic antigens, such as CD45 (ref. 15). Using the same system, it was shown that mechanical shear stress enhances the formation of blood cells by inducing the formation of CD41, c-Kit-positive cells that produce increased levels of nitric oxide (NO) and upregulate Runx1 and Myb[16]. Similar observations were also made in zebrafish, demonstrating the need for blood flow and NO production for haematopoietic stem cell formation[17].

with classic 'forward' differentiation. This is where the fields of induced lineage conversions and developmental biology merge: we propose that the cell interconversions elicited experimentally by transcription factors may mimic specific physiological cell fate transitions and that the two processes are fundamentally similar.

In this review we briefly chart the evolution of the transcription factor perturbation experiments and discuss how they have provided fundamental insights into the process of lineage specification. They identified lineage-instructive regulators, revealed the principle of transcription factor cross-antagonisms in binary lineage decisions and helped explain the dynamic behaviour of regulatory networks. We also discuss more generally what lineage reprogramming has shown about mechanisms of development (see also ref. 18) and place them in the context of emerging epigenetic landscape models. Finally, we compare transcription-factor-mediated lineage reprogramming to iPS cell reprogramming and discuss their potential for regenerative therapy.

Charting the beginnings

The instructive role of transcription factors in lineage specification was demonstrated in the 1980s, when Harold Weintraub's laboratory discovered that forced expression of MyoD can induce myotube formation in a fibroblast cell line[2] (Fig. 1a). Evidence for the reciprocal regulation of lineage-restricted genes came from the blood system, which, with its diversity of well-defined cell lineages and prospectively isolatable intermediate progenitors, is an ideal venue for lineage-conversion experiments. Thus, when ectopically expressed in cell lines of monocytes (macrophage precursors) at high levels, the erythroid-megakaryocyte-affiliated transcription factor GATA1 not only induced the expression of erythroid-megakaryocyte lineage markers, but also downregulated monocytic markers[3,19]. Lower levels of GATA1 induced the formation of eosinophils, in line with its levels in normal eosinophils[3] (Fig. 1a). The monocytic to erythroid switch could also be effected in the opposite direction: expression of PU.1 (also known as Sfpi1) in an erythroid-megakaryocytic cell line induced its conversion to the monocytic lineage, repressing GATA1 (ref. 20). A potential caveat of these studies was their reliance on cell lines, which may be more inherently plastic than their normal counterparts. This objection was dismissed when ectopic expression of GATA1 produced erythroid-megakaryocytic-eosinophil-basophil output from granulocyte-macrophage progenitors freshly isolated from normal bone marrow[21]. More recently it was shown that even fully differentiated cells can be switched: C/EBPα, a transcription factor required for the formation of granulocyte-macrophage precursors[22] can convert committed B- and T-cell progenitors into functional macrophages at frequencies approaching 100%[23,24]. Mature immunoglobulin-producing B cells could also be switched, although at lower frequencies[23] (Fig. 1d).

Mechanistic implications of the GATA1:PU.1 paradigm

The high efficiency of induced lineage reprogramming in the blood system indicates that ectopically expressed transcription factors interact with endogenous components of the recipient cells' transcriptional network. The switching mechanism may therefore encapsulate the principles of normal lineage specification. Indeed, the dominance of either GATA1 or PU.1 represents one of the earliest and most fundamental decisions during haematopoietic development, serving as a paradigm for cross antagonistic transcription factor interactions[25,26]. In Fig. 3 we have extrapolated the PU.1:GATA1 antagonism to normal lineage specification by assuming that basic gene expression programs of monocytic and erythroid cells are directly controlled by PU.1 and GATA1, respectively. PU.1, and possibly GATA1, also controls its own expression, forming an auto-regulatory loop[27,28]. The model is reminiscent of a simpler genetic switch controlling the choice between lysogenic and lytic pathways in phage lambda by the cross-antagonistic and autoregulatory transcriptional regulators Cro and C1[29]. The central role of transcription

Studies of GATA1-mediated myelomonocytic cell fate conversions showed that it directly binds to PU.1 protein (reviewed in ref. 31). High levels of GATA1 inhibit PU.1 by displacing c-Jun, a cofactor of PU.1, thus leading to the collapse of the monocytic program[33]. Conversely, PU.1 expressed in erythroid precursors interacts with GATA1 bound to promoters of target genes, including α- and β-globin as well as *EKLF* (also known as *KLF1*), and converts an activating into a repressive complex through displacement of the coactivator CREB-binding protein (CBP) and recruitment of the retinoblastoma protein[34] (Fig. 3d). Therefore, lineage-instructive transcription factors not only 'step on the accelerator' to induce a new gene expression program, but also 'put on the brakes' to inactivate key regulators of alternative cell types, leading to extinction of markers characteristic of the old phenotype. Once one of the two factors has become dominant the conflict is resolved and commitment ensues.

Transcription factor ablation and lineage re-specification

If forced resolutions of transcription factor cross antagonisms specify lineages it should also be possible to trigger differentiation by loss of transcription factor function. Evidence gathered in the haematopoietic system supports this prediction. The earliest haematopoietic cells in zebrafish arise anteriorly as macrophage precursors and posteriorly as erythroid precursors. Morpholino-mediated knockdown of PU.1 leads to the ectopic formation of haemoglobin-producing cells in the dorsal region[35] whereas inactivation of GATA1 induces the formation of monocytic cells in the posterior region[36]. This indicates that committed erythroid and monocytic progenitors can be re-specified when the opposing key regulator is ablated. However, recent work indicating pluripotency of the posterior population indicates a more complex PU.1/GATA1 balance in this region[37]. Inactivation of key regulators may also lead to the reactivation of earlier genetic programs in committed cells, resulting in their dedifferentiation and activation of multilineage potential. For example, ablation of Pax5 in B-cell precursors activates expression of genes from alternative haematopoietic lineages. Under appropriate culture conditions or after transplantation these cells can differentiate into granulocyte/macrophage, T-cell, dendritic, natural killer and osteoclast lineages[38]. Alternative lineage potentials can even be resuscitated in fully functional B cells: transplantation of Bcl2-stabilized Pax5-deficient cells into immunodeficient mice generates T cells, which contain immunoglobulin rearrangements[39] (Fig. 1e). This conversion does not appear to be direct as it entails the dedifferentiation to a lymphoid precursor.

Influence of cell-extrinsic signals

So far cross-antagonistic switches were presented as relatively simple circuits functioning in a broadly cell-intrinsic manner. In reality, most antagonistic circuits in metazoa are subject to graded external inputs[40,41]. An example that illustrates the interplay between cell intrinsic and extrinsic signals is relevant for the branching of CD4^{+} T lineages into T_H17 and T_{reg} type helper cells[42]. Differentiation of T_H17 cells requires RORγt whereas T_{reg} cells require Foxp3, transcription factors that are coexpressed in naive CD4 cells. The differentiation of these two cell types is orchestrated by a transforming growth factor (TGF)-β gradient. Low TGF-β concentrations plus interleukin (IL)-6 and IL-21 upregulate RORγt and promote the formation of T_H17 cells. In contrast, high TGF-β concentrations upregulate Foxp3 and facilitate T_{reg} cell formation. In addition, Foxp3 inhibits RORγt function, probably through direct protein interaction[42]. Recent experiments have shed light on the long-standing debate as to whether or not haematopoietic cytokines have a lineage-instructive function or merely promote survival and proliferation of already committed cells. These experiments, conducted with isolated bipotent progenitors and followed by time-lapse microscopy, indicate that myeloid cytokines are capable of instructing lineage choice[43]. Similarly, experiments with mice deficient for the transcription factor MafB point to an instructive role for the macrophage colony stimulating factor (M-CSF) during myeloid commitment of haematopoietic stem cells; in the absence of
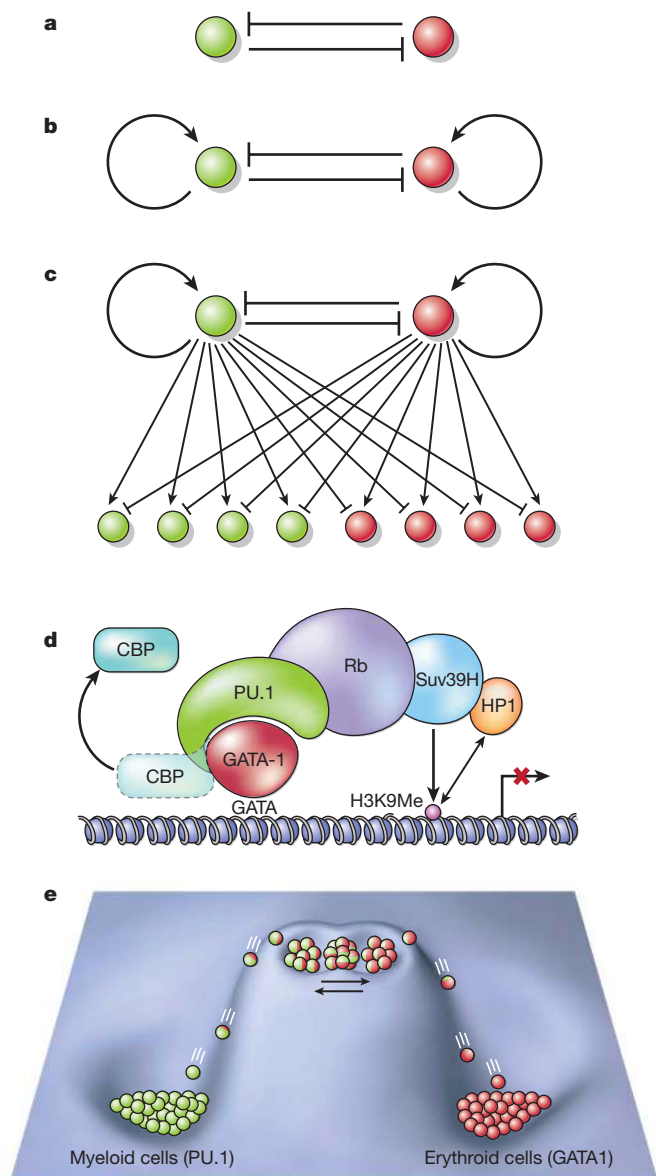
Figure 3 | Transcription factor cross-antagonism: the PU.1:GATA1 paradigm. a, In the simplest formulation of cross-antagonism, the two regulators (represented as green and red spheres, respectively) negatively influence each other. **b,** Representation of a cross-antagonistic motif in which the transcription factors also autoregulate. **c,** Here the two factors are shown to positively or negatively regulate the repertoire of their own and each other's target genes. **d,** Scheme of the biochemical mechanisms that underlie the GATA1 arm of the PU.1:GATA1 antagonism. To activate a target gene in erythroid cells GATA1 recruits the histone acetylase CREB-binding protein. Overexpressed PU.1 displaces CREB-binding protein (CBP) by binding to GATA1 and recruits Rb as well as Suv39H protein. This results in methylation of lysine 9 in histone H3 and recruitment of HP1a, causing repression of the target gene[34]. **e,** Representation of the PU.1:GATA1 antagonism as a binary attractor model in a modified Waddingtonian epigenetic landscape. Bicoloured marbles in the upper, shallow basin represent monocytic/erythroid progenitors that express different ratios of PU.1 and GATA1. These progenitors fluctuate between different states determined by the relative amount of PU.1 and GATA1. Cells at both ends of the spectrum are biased towards either monocytic or erythroid differentiation. During spontaneous or induced commitment they move out of the basin and roll into the attractor basins below. Green marbles represent monocytic cells expressing high levels of PU.1; red marbles erythroid cells expressing high levels of GATA1.

factor cross antagonisms in binary cell fate choices[30–32] is the single most important concept that has emerged from lineage reprogramming experiments.

MafB haematopoietic stem cells become hyper-responsive to M-CSF through activation of the M-CSF receptor regulator PU.1, resulting in an enhanced myelomonocytic output after transplantation[44].

Interactions between external inputs and cell fate decisions are not restricted to blood cells. A classical example is the interplay between an activin gradient and the transcription factors brachyury, goose-coid and Mix during patterning of mesoderm. Brachyury, which autoregulates its own production, is activated by low and high levels of activin (nodal) signalling, leading to different developmental out-comes. Low levels lead to the activation of brachyury and repression of goosecoid through inactivation of Mix, resulting in the production of posterior mesoderm. High activin levels induce Mix expression, which in turn represses brachyury by activating goosecoid, resulting in endoderm and anterior mesoderm. As predicted, loss of goosecoid results in the production of posterior mesoderm at the expense of anterior mesoderm and endoderm[45].

Transcription factor network assembly and lineage outcome

Lineage switching experiments in the haematopoietic system have shown that the order in which two transcription factors become expressed in a progenitor can decide lineage outcome. Using prospec-tively isolated common lymphoid progenitors, sustained expression of C/EBPα generates granulocyte-macrophages whereas sustained expression of GATA2 generates mast cells. However, two entirely new cell types, eosinophils and basophils, are generated when CEBPα and GATA2 are sequentially expressed and in a different order[46] (Fig. 4). This shows that the same transcription factor pair can specify alternative cell types, probably because the separate expression of C/EBPα and GATA2 generates two distinct intermediate progenitors whose fates are further redirected by the incoming factor. It is possible that C/EBPα and GATA2 interact with different co-regulators in different cell types. Such a sequential participation of transcription factors in different protein complexes during differenti-ation has been likened to the changing interactions of guests at a cocktail party[47]. These observations underscore the importance of timing and cell context for the assembly of cell type specific transcription factor networks.

Gene regulatory networks and cell fate attractors

A popular framework for conceptualizing the specification of differ-ent cell types is that of the epigenetic landscapes proposed by
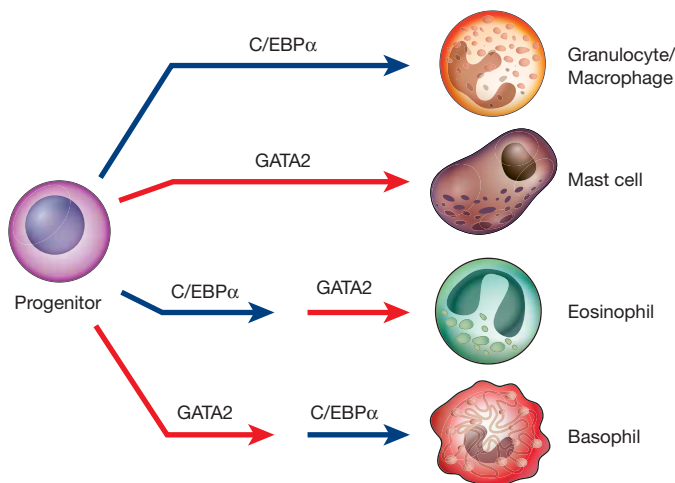

Figure 4 | Timing of transcription factor expression and lineage outcome. Forced expression of C/EBPα in common lymphoid progenitors induces the formation of granulocytes and macrophages, whereas GATA2 induces the formation of mast cells. If C/EBPα expression is followed by GATA2 the cells turn into eosinophils. If the order of expression is reversed they become basophils (after ref. 46). Similar rules apply to the physiological specification of the relevant cell types from the multipotent myeloid progenitor[26].

Waddington[48]. Extrapolating from Waddington, different cell types may be seen as stable solutions of transcription factor networks—or 'attractors'—which occupy the basins of Waddington's land-scape[49–51]. Within this framework developmental intermediates, such as multipotent progenitors, may be viewed as representing metastable states that are characterized by co-expression of cross-antagonistic regulatory factors driving alternative lineage-affiliated programs of gene expression. This arrangement affords structuring of lineage choice and ensures robustness of the differentiated state. Robustness may also underlie why direct reprogramming by an ectopic transcrip-tion factor works so well: it only requires destabilization of one stable network solution and the realization of another stable solution, a transition that can probably be achieved through multiple paths.

These views square well with experiments indicating that multi-potential cells prime competing lineage-affiliated gene expression programs before commitment—a phenomenon dubbed 'lineage priming'[52–54]. Mostly on the basis of work with a bipotent haemato-poietic cell line it has further been proposed that all cells within the multipotential compartment are not equivalently primed and may fluc-tuate between different lineage-biased states[55,56] (Fig. 3e). Whether these fluctuations, which have also been observed for Nanog expression within self-renewing ES cells[57], are driven by 'noise' or by other cell-intrinsic mechanisms is not known.

In complex differentiation hierarchies, like those of the blood system, one can envisage cell states cascading down the valleys of a mountain range, with each bifurcating decision heralded by activa-tion of new cross-antagonistic pairings, which themselves result from the outcome of a prior 'bout' (Fig. 5). Although the combination of cross-antagonistic and autoregulatory circuits can in principle con-vert small initial asymmetries within cells into stable or metastable network states representing distinct cell types[58–60], the particular cross-antagonistic circuit used to select choice may still be amenable to resetting in the other direction. As cells cascade through bifurc-ating decisions, prior switches become less available, decreasing the probability of reversal and further restricting possible cell-type solu-tions going forward[58]. This 'passing of the baton' of networks from one cell-state to the next ensures forward momentum during lineage specification in development and explains the temporal and cellular profiles of transcription factor activity[61]. Some of the aforementioned antagonisms best exemplify such a mechanism. At the level of the common myeloid progenitor (CMP), resolution of the PU.1:GATA1 antagonism leads to the bifurcation into bipotential granulocyte/ macrophage progenitors (GMP) and megakaryocyte/erythroid pro-genitors (MEP). In turn, at the level of these bipotent precursors resolution of the Gfi1:Nab2/Egr antagonism in granulocyte/macro-phage progenitors and the EKLF:FLI1 antagonism in megakaryocyte/ erythroid progenitors creates four distinct cell types: granulocytes, macrophages, erythrocytes and megakaryocytes[62,63]. Another example is the sequential cross-antagonisms during T-cell development, first involving GATA3 and T-bet at the level of T helper type 1/T helper type 2 (Th1/Th2) precursors and then RORγt and Foxp3 at the level of naive CD4 T helper cells[42,64]. This 'branching compartmentalization' allows decisions to be inherited from one progenitor to the next as well as effecting a separation of states, affording the re-use of a transcrip-tion factor in a different network context. Such a modus operandi accommodates the observations that the order of expression of tran-scription factors may affect cell fate outcomes and that the same final cell states can be reached via alternative routes, as exemplified by the different origins of granulocyte-macrophage precursors (Fig. 5).

Within the landscape/attractor-based conceptual framework, it is easy to see how cell fate transitions, such as those exhibited by lineage reprogramming events, may be favoured between cell types closely connected through shared regulatory switches. It also predicts that the efficiency by which transcription factors induce lineage conver-sions depends on the proximity of the cell type in question and that bridging greater distances may require additional factors acting at earlier common branch points. A possible example is the observation
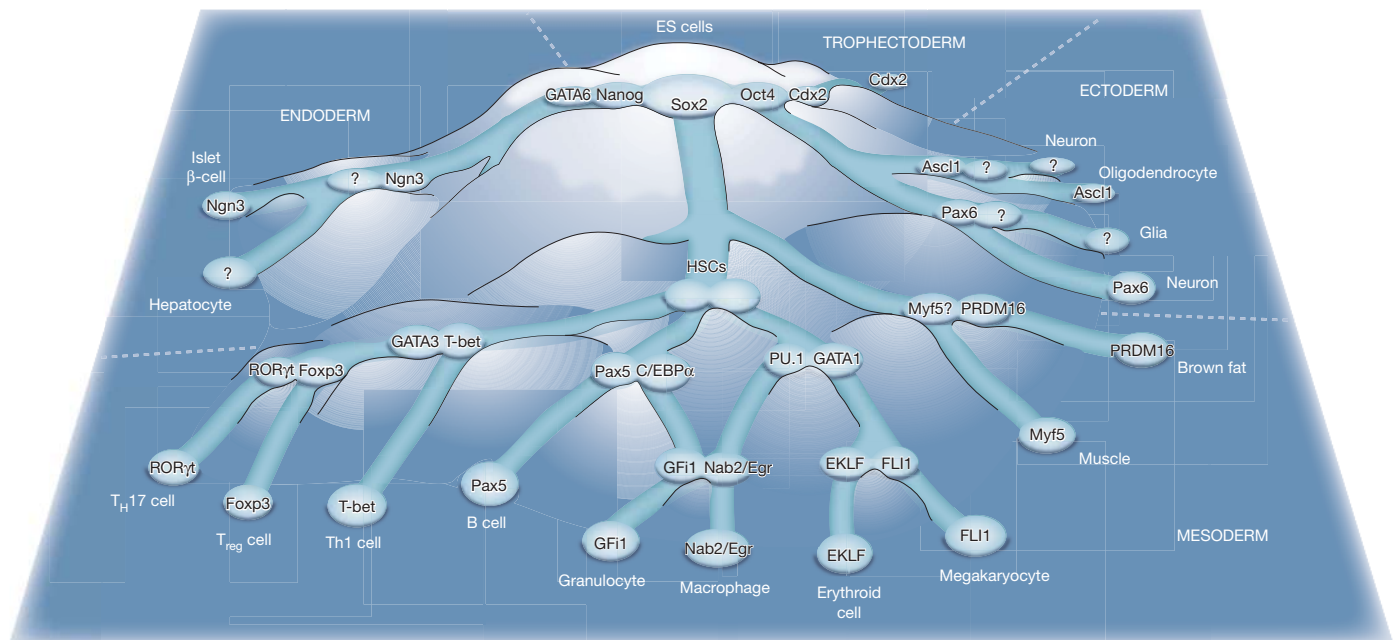
Figure 5 | Transcription factor cross-antagonisms in a cascading landscape of unstable and stable cell states. The territory, represented as a mountain range, depicts all possible solutions of a single regulatory network that specifies cell identity. Robust network states correspond to stably differentiated cell types (deep basins in the low-lying plains) whereas unstable solutions correspond to ridges and slopes in the landscape. The latter are only fleetingly occupied during development and thus unlikely to correspond to observable cell types. The route between pluripotent and fully differentiated network states is punctuated by a series of metastable states corresponding to progenitors characterized by the cross-antagonistic interaction of competing lineage-affiliated transcription factors. Within these goggle-shaped 'binary attractors' transcriptional networks fluctuate between lineage-biased states before exit either into a stable attractor

corresponding to a developmental endpoint, or into a subsequent metastable attractor where a secondary lineage decision is taken. In this model the sequential establishment and resolution of transcription factor cross-antagonisms is a driving force in lineage specification. However, such a mechanism might not apply to earlier intermediates, which may only be partly restricted and do not necessarily commit through simple binary decisions. The intermediates and paths depicted may not be exclusive or obligatory transit points, but rather represent the most favoured possibilities. Although all the transcription factors shown have been experimentally demonstrated to possess lineage-instructive capacity, their precise mechanism of action or the identity of a presumed antagonistic partner (indicated with a question mark) is not known. ES cells, embryonic stem cells; HSCs, haematopoietic stem cells.

that switching into β-cell islets of hepatic progenitors only requires Ngn3 (ref. 65), whereas switching of exocrine pancreas cells requires in addition Pdx1 and MafA[66] (Fig. 1e). Transcription-factor-mediated lineage conversions may thus be achieved by effecting the same regulatory interactions that drive normal differentiation. However, the actual path taken by the cells is not clear. Here, two possibilities can be considered: (1) the ectopic transcription factor first resets the cell's regulatory network to an earlier branch point position and then directs it back along a physiological trajectory to the new cell type; (2) alternatively, reprogramming results in direct crossing of the 'ridge' that divides the two lineage-committed territories without reactivating progenitor programs.

Generalizing transcription factor cross-antagonisms

Many binary junctures during development seem to be governed by cross-antagonistic transcription factor interactions. As summarized in the epigenetic landscape in Fig. 5, the haematopoietic system offers the largest number of well-studied pairs. This may simply reflect the wealth of knowledge of developmental intermediates in which cross-antagonistic interactions may be studied. Alternatively, perhaps because of their largely free-floating nature, haematopoietic cells might be more weighted towards cell-autonomous decisions. In addition to the examples already discussed, the decision of erythroid against megakaryocytic cells is effected by the balance of EKLF:FLI1 (refs 63,67); granulocytes against macrophages by Gfi1:Nab2/Egr[62]; erythroid-megakaryocyte precursors against eosinophils by C/EBPb:FOG-1 (ref. 68); Th1 against Th2 cells by T-bet:GATA3 (ref. 64); and T_H17 against T_{reg} cells by RORγt:Foxp3 (ref. 42). A

potential cross-antagonism outside the haematopoietic system is played out within skeletal muscle and brown fat precursors. Here, enforced expression of PRDM16 in Myf5-expressing mesenchymal progenitors induces their differentiation into brown fat cells. Conversely, inactivation of PRDM16 in these cells promotes muscle differentiation and causes a loss of brown fat characteristics[69]. This suggests that PRDM16 controls a bidirectional fate switch between skeletal myoblasts and brown fat cells. Other examples include the conversion of astrocytes into neurons[70]; neural precursors into oligodendrocytes by Ascl1 (ref. 71); neural precursors into inner ear sensory cells by Atoh1 (ref. 72); liver cells into islet β-cells by Pdx1-VP16 (ref. 73); and hepatocyte precursors into insulin producing islet-like β-cells by Ngn3 (ref. 65). Of note, for none of these factors has antagonistic partners been described, raising the possibility that in the absence of a lineage-instructive transcription factor the relevant precursors enter a default pathway. The very first developmental decisions in the pre-implantation embryo also seem to be guided by transcription factor cross-antagonisms. Here, the best-studied example is the pair Cdx2:Oct4, where forced expression of Cdx2 in ES cells induces the formation of trophectoderm cells by inhibiting Oct4 through direct protein interaction[74]. Finally, the predominance of either Nanog or GATA6 decides whether ES cells maintain their identity or differentiate into endoderm[75].

The new world order of iPS cells

So, how then does this framework of induced lineage reprogramming, normal forward differentiation and developmental transdifferentiation relate to the induced conversion of somatic into embryonic

stem cells—the new world order of iPS cells? Before tackling this question, let us first consider the salient features of the iPS situation. Initial experiments demonstrated that the combination of Oct4, Sox2, Klf4 and Myc can induce the transition from fibroblasts into stable self-renewing cells closely resembling ES cells[8] (Fig. 1c). iPS reprogramming could subsequently also be achieved with a range of somatic cell types, including differentiated cells such as hepatic[76] or islet β-cells[77]. All of these cells express their own cell-type-specific repertoires of lineage-instructive transcription factors. How does reprogramming proceed in these cases? It seems unlikely that iPS reprogramming factors have evolved to interact with the large variety of lineage-affiliated transcription factors and thus divert regulatory networks within cells that are many branch points away from the pluripotent state. Instead, the low frequency and long duration of iPS reprogramming, in excess of a week (reviewed in refs 78, 79), suggests that stochastic mechanisms are involved and that several rounds of cell divisions are required.

As for directly induced lineage conversions, it would be predicted that reprogramming of cells that are developmentally closely related require fewer transcription factors. Indeed, neural progenitors, which already express Sox2, Klf4 and Myc, can be turned into iPS cells with only Oct4 (ref. 80). However, their reprogramming efficiency remains exceedingly low, suggesting that even here stochastic processes are at play. How reprogramming works remains unclear[79]. Oct4 and its partners might gradually gain access to hidden DNA binding sites through the dynamic 'breathing' of chromatin, eventually upregulating the corresponding endogenous factors and thus establishing transgene independence by activating autoregulatory loops. Another mechanism is the direct interaction with chromatin-remodelling proteins, leading to upregulation of critical ES cell regulators such as Nanog[81]. Repression of the resident cells' program in turn might be mediated by the capacity of ES cell regulators to actively silence differentiation-affiliated transcription factors, such as through recruitment of polycomb complexes and formation of bivalent chromatin domains[82].

No matter what the relevant mechanisms are, ES cells, and by inference iPS cells, are unique in that they represent a cellular ground state whose default configuration is that of self-renewal[83]. This ground state is particularly accessible to the transcription factor program that establishes the highly stable Oct4–Nanog–Sox2 network, where the three factors regulate each other's expression as well as their own, in an arrangement known as a fully connected triad[84–86]. The high proliferative potential of ES/iPS cells and the stability of the triad may explain why the relatively rare iPS reprogramming events can so easily be trapped in culture. Because of the heterogeneity of ES cells[57] the ES cell state can be seen as a broad attractor in which many pluripotent network configurations may co-exist and interconvert along the lines discussed for the blood system[56,87]. This level of tolerance in possible network configurations may additionally increase ease of access from many if not all somatic cell states.

Induced lineage reprogramming and regenerative medicine

The question then arises whether, given enough knowledge, it will be possible to directly reprogram any cell type into another and to custom-design cells for regenerative therapy from easily obtainable cell sources. Consider B cells for example: we know that transcription factor gain or loss of function can convert these cells into macrophages as well as into T cells and ES cells. But can B cells be induced directly to become, say, haematopoietic stem cells or islet β-cells at high frequencies, effecting 'long jumps' within the landscape of Fig. 5? Attempts to induce direct transitions between distantly related somatic cell types have been inconclusive so far. For example, forced expression of MyoD in a keratinocyte cell line did not induce myotube formation and only upregulated a few mesenchymal genes[88]. Co-expression of PU.1 and C/EBPα in fibroblasts converted them into macrophage-like cells, but the resulting cells were only partially functional[89]. Most encouraging are results describing the induction

of the rapid and extensive reciprocal regulation of keratinocyte- and muscle-associated genes in heterokaryons between human keratinocytes and mouse muscle cells. Here, phenotypic dominance could be achieved by increasing the ratio of one cell type over the other[90]. It will now be interesting to see whether efficient long jumps can be achieved with defined genes into cells closely resembling their normal counterparts. However, for regenerative purposes a full equivalence to normal cells may not be necessary as long as the induced cells perform the desired functions *in vivo* and long term.

In conclusion, it may eventually be possible to generate cells 'a la carte' by forced transcription factor expression in cultured biopsies. However, because most progenitors and differentiated cells do not proliferate, the cells generated probably cannot be expanded, as is possible with iPS cells. For cell replacement therapy purposes it is therefore crucial that high frequency transitions can be achieved. This might necessitate, in addition to simple overexpression of transcription factor(s), a whole arsenal of tricks, including the inducible, sequential and graded expression of transcription factors[46,91], transcription factor knockdowns[38,92], modulation of microRNAs and chromatin remodelling factors[93–95], or treatment of the cells with chemicals[96]. If successful, such experiments, aside from their clinical potential, would provide valuable information about the regulatory networks that specify different cell types. A promising alternative for the directed induction of desired cell types are *in vivo* approaches. Perhaps the most spectacular example to date is the conversion of exocrine pancreas cells into fully functional islet β-cells in mice by Pdx1, Ngn3 and MafA[65,66]. In spite of these successes, custom-designing cells for cell therapy in humans is still a long way off. Only time will tell whether it will prevail over the application of cells derived from iPS or ES cell lines. But it seems safe to predict that transcription-factor-induced cell reprogramming will continue to reveal hidden secrets of cell differentiation for a long time to come.

Note added in proof: Two new examples of transcription factor cross antagonisms outside the blood cell system have recently been described. One describes the antagonism between the Ngn3 target Arx and Pax4 in pancreas development[97]. The other is an antagonism playing out during vascular and muscle specification in the dermomyotome[98].

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