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Transdifferentiation—Fact or Artifact

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Abstract Normal development appears to involve a progressive restriction in developmental potential. However, recent evidence suggests that this progressive restriction is not irreversible and can be altered to reveal novel phenotypic potentials of stem, progenitor, and even differentiated cells. While some of these results can be explained by the presence of contaminating cell populations, persistence of pluripotent stem cells, cell fusion, etc., several examples exist that are difficult to explain as anything other than ''true transdifferentiation'' and/or dedifferentiation. These examples of transdifferentiation are best explained by understanding how the normal process of progressive cell fate restriction occurs during development. We suggest that subversion of epigenetic controls regulating cell type specific gene expression likely underlies the process of transdifferentiation and it may be possible to identify specific factors to control the transdifferentiation process. We predict, however, that transdifferentiation will not be reliable or reproducible and will probably require complex manipulations. J. Cell. Biochem. 88: 29–40, 2003. Published 2002 Wiley-Liss, Inc.†

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RESTRICTION IN CELL FATE

A general operating assumption in the development of the nervous system, as in other systems, is the idea of a progressive restriction in developmental potential. More differentiated cells have a more limited repertoire of fate choices and fully differentiated cells do not have any alternative fates and may not be able to reenter the cell cycle at all. Embryonic stem (ES) cells are totipotent and can generate every cell type in the body while tissue specific stem cells, on the other hand, are restricted to developing cells of that germ layer or organ type. Tissue or germ layer specific stem cells undergo further developmental restrictions generating cells that are capable of cell division and at least limited self-renewal but are unipotent, bipotent, or more restricted in their developmental profile than stem cells of that particular tissue or organ. These cells have been termed blast cells, transit amplifying cells, or intermediate precursor cells. Several stages of such progressively, more restricted intermediate precursors may exist which will ultimately give rise to fully differentiated cells (reviewed in [Rao, 1999; Weissman et al., 2001]). Fully differentiated cells generally are mitotically inactive and may be capable of reentering the cell cycle upon appropriate stimulation (transient G0 arrest) or may be incapable of cell cycle reentry (permanent G0).

The development of the hematopoietic system and nervous system are good examples of such progressive, developmentally restricted differentiation (Fig. 1). Neural stem cells (NSCs), which are distinct from even more pluripotent ES cells, generate all cells within the nervous system. Such NSCs have been identified from multiple species, can undergo self-renewal, and retain the ability to differentiate into neural crest, neurons, astrocytes, and oligodendrocytes even after prolonged periods in culture. NSCs generate more restricted precursors and several such restricted precursor cells have been

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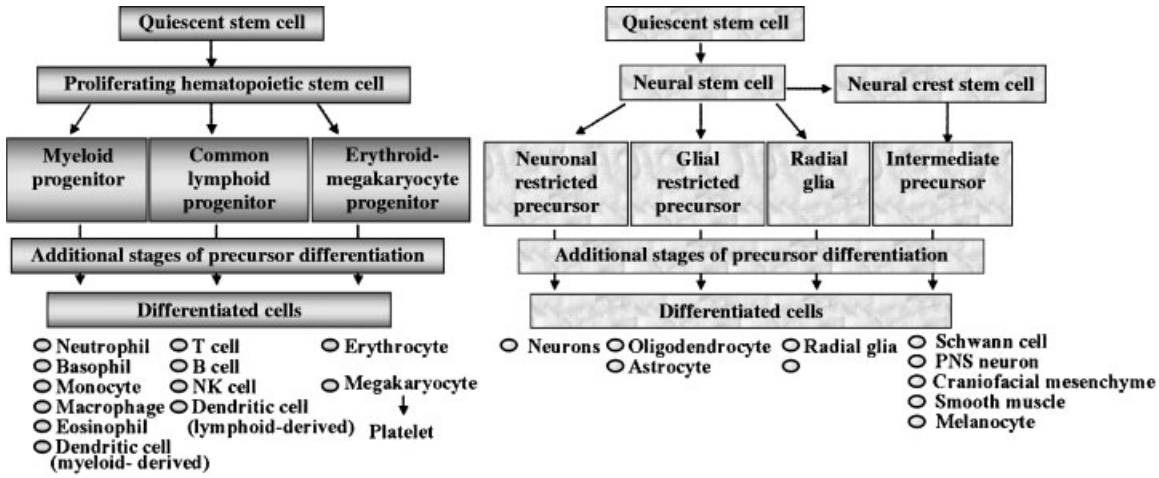

Fig. 1. Progressive restriction in developmental potential. Two examples of cell fate restriction during development are provided. Both models represent summaries and are not meant to describe the complete lineage in either the nervous or hematopoietic system.

identified (Fig. 1). Restricted precursors differ in cytokine response, self-renewal ability, and the repertoire of differentiation from NSCs. This difference is maintained after in vitro culture and transplantation into regions, where such cells are normally not present. Restricted precursors generate fully differentiated cells that include postmitotic neurons and glia (oligodendrocytes and astrocytes). Glial cells, while quiescent, can reenter the cell cycle while postmitotic neurons are thought to be held in permanent G0 arrest.

Such a model of progressive restriction in cell fate is neither unique nor was it first enunciated as an explanation for nervous system development. Stem cells and more restricted precursor cells have been identified in multiple tissues including bone marrow, liver, pancreas, and skin (reviewed in [Cai and Rao, 2002]) and similar hierarchal models of progressive restriction in developmental fate have been proposed in most organ systems. Indeed, as early as 1960, a hierarchal model of bone marrow stem cell differentiation had been established.

The evidence that cells are more restricted in their developmental potential has been tested in back transplant (heterochronic) experiments or side-by-side analysis in a variety of systems. In most cases overwhelming evidence has shown that restriction in developmental potential occurs relatively early in embryological development and in most instances, is not readily reversible even when cells are transplanted into a permissive environment. These and other experiments have suggested that progressive restriction of developmental potential is a normal aspect of development and that phenotypic plasticity is uncommon. The idea that there is a cell intrinsic change that restricts the potential of initially pluripotent cells is appealing as it helps explain how the same regulatory molecules can be reiteratively used at multiple stages and in different tissues to direct differentiation and different fates in multiple distinct lineages (reviewed in [Weissman et al., 2001]).

PLASTICITY

While in vivo development appears to follow a sequential pathway of progressive fate restriction, several lines of evidence suggest that differentiation may not be entirely a one-way street. Multiple investigators have shown that tissue specific stem cells, intermediate precursors, and even fully differentiated postmitotic cells can be induced to alter their phenotypic profile in dramatic ways [Tosh and Slack, 2002]. Such plasticity has been described in ectodermal, endodermal, and mesodermal progenitors and differentiated cells (Table I).

Cells may follow multiple pathways to acquire a distinct phenotype. Cells may revert to an earlier, more primitive phenotype that now would have a wider differentiation potential. Thus a neuronal restricted precursor may lose its developmental restriction and revert to a NSC; it then becomes capable of differentiating into glial cells. Such a reversion to a developmentally

TABLE I. Plasticity of Precursors of Three Germ Layers

Precursors	Transdifferentiation	Reference
Neural stem cell	Hematopoietic cell	[Bjornson et al., 1999]
VENT cell	Muscle cell	[Ali et al., 1999]
Neural cell	Bone and soft tissue	[Franchi et al., 2001]
Skin cell	Neuron	[Toma et al., 2001]
Hematopoietic cell	Microglia and macroglia	[Eglitis and Mezey, 1997]
Hematopoietic cell	Lung alveolar epithelium	[Kotton et al., 2001]
Hematopoietic cell	Myocardium	[Orlic et al., 2001a]
Muscle cell	Hematopoietic cell	[McKinney-Freeman et al., 2002]
Muscle cell	Adipocyte	[Hu et al., 1995]
Hepatocyte	Pancreatic islet cell	[Overturf et al., 1997]
Pancreatic islet cell	Hepatocyte	[Shen et al., 2000]

more immature phenotype would be considered an example of dedifferentiation (Fig. 2). Dedifferentiation essentially retraces the steps followed during normal phenotypic differentiation.

Alternatively, cells may acquire the capability to differentiate into a unique or novel phenotype that does not involve going through a developmentally more immature phenotype (stage). For example, if a postmitotic neuron acquires the ability to generate glial cells without first reverting to a recognizable, undifferentiated, progenitor state, then one would consider it an example of transdifferentiation rather than dedifferentiation. Cells may transdifferentiate from one cell type to another within the same tissue (glia to neuron, e.g.) or into a completely different tissue derivative (neuron to hematopoietic cell, e.g.).

Both dedifferentiation and transdifferentiation should be distinguished from competence and the normal fate of cells (reviewed in [Tsonis, 2000]). Cells may be competent to differentiate into a particular phenotype but this competence may not have been recognized, or not be expressed during normal development (absence of cues) or be actively repressed and this fate may be readily revealed by altering the environment. Such demonstration of competence is not evidence for transdifferentiation or dedifferentiation. For example, the normal fate of radial glia appears to be to differentiate into astrocytes. This has, on occasion, been called transdifferentiation, but in our minds should be considered a normal fate of the radial glial cell. Likewise, neural crest cells are capable of differentiating into bone cartilage and muscle, but trunk crest does not normally contribute to such tissue. This capacity, however, can be readily demonstrated in culture and in our minds is not evidence for transdifferentiation, but rather reflects the unrevealed competence of neural crest cells.

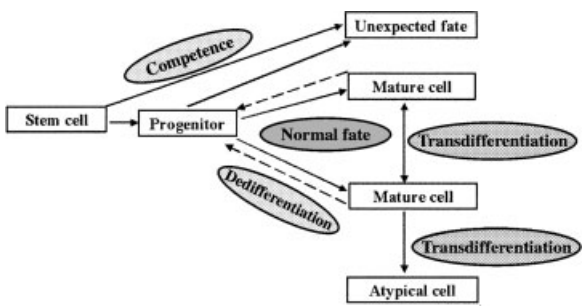

Fig. 2. Competence, fate, dedifferentiation, and transdifferentiation. True transdifferentiation must be distinguished from the normal competence of the cells. Dedifferentiation and transdifferentiation may have the same ultimate endpoint but it is still useful to distinguish between the two, if possible. Harnessing the ability of cells to generate atypical phenotypes will require different approaches depending on whether the cell reverted to an earlier more primitive phenotype or transformed via an atypical pathway.

Distinguishing dedifferentiation from transdifferentiation is not easy, especially in systems where normal development is not well characterized. In general, if the cytokine requirement and sequence of markers expressed recapitulate those detected during the normal pathway of differentiation or if markers characteristic of an earlier precursor cells can be identified during differentiation, then one would consider this a dedifferentiation process. If, however, the transition is rapid, does not follow a normal sequence, or cannot be readily explained by our understanding of the normal sequence of development, then one would consider this a transdifferentiation process. Acquisition of neural markers by mesenchymal cells upon exposure to dimethylsulfoxide (DMSO) [Woodbury et al., 2000] may be a transdifferentiation event, while the presence of neural and connective tissue elements in Ewings sarcoma [Franchi et al., 2001] may be an example of dedifferentiation.

While dedifferentiation and transdifferentiation have been described in the past [Yasuda et al., 1989; Reh and Pittack, 1995; Bosco et al., 1997], there has been little concerted effort to analyze the process in any detail. However, there has been a renewed interest in recent years as this process may allow us to obtain a source of cells for tissue replacement strategies in diseases, where stem cells are limiting. Much of the excitement has focused on the ability of two populations of cells to transdifferentiate: neural cells, because they can be maintained in culture for prolonged time periods; and bone marrow stem cells, because they can be obtained in large numbers from adult tissue with relative ease [Clarke and Frisen, 2001]. For example, bone marrow stem cells obtained for autologous transplant from adult tissue could be transdifferentiated into dopaminergic neurons that could be used to treat Parkinson's disease [Azizi et al., 1998]. Given that getting NSCs to differentiate into dopaminergic neurons is difficult [Ling et al., 1998; Potter et al., 1999], transdifferentiated autologous bone marrow cells are an attractive alternative to limited heterologous fetal tissue cells. Demonstrating transdifferentiation and identifying the factors regulating the process of transdifferentiation into neural tissue or any tissue where obtaining therapeutic numbers of tissue specific stem cells is difficult has become a major area of research.

In recent years, reports on the number and variety of cells that transdifferentiate has been quite startling and it is somewhat surprising that one sees any fate restriction in tissue at all. Recent studies have shown NSCs when transplanted in the bone marrow of irradiated mice will generate hematopoietic derivatives [Bjornson et al., 1999] and when injected into the blastocyst, will generate cells in all three germ layers [Clarke and Frisen, 2001]. Likewise, mesenchymal stem cells (MSCs) and bone marrow cells will generate astrocytes and possibly neurons when infused into the brain [Eglitis and Mezey, 1997; Kopen et al., 1999], pneumocytes when infused into the lung, and cardiac myocytes in models of infarction [Orlic et al., 2001a,b]. Hepatocytes may be capable of differentiating into pancreatic islet cells and vice versa [Overturf et al., 1997; Shen et al., 2000]. Skin cells may be capable of differentiating into neurons [Toma et al., 2001], and muscle precursor cells may be capable of differentiating into bone marrow as well (reviewed in [Tosh and Slack, 2002]). These and many other transtissue differentiation results ([Jackson et al., 1999; Petersen et al., 1999]; reviewed in [Tosh and Slack, 2002]) suggest that at least tissue specific stem cells may be more pluripotent than previously thought or still retain the ability to dedifferentiate.

Other investigators have examined the property of still more restricted populations of cells to dedifferentiate or transdifferentiate and, somewhat surprisingly, have noted that the ability to transdifferentiate is not restricted to stem cell populations. In the central nervous system (CNS) oligodendrocyte precursors, astrocytes, and radial glia have all been shown to be capable of dedifferentiating/transdifferentiating into mature neurons [Kondo and Raff, 2000; Laywell et al., 2000; Malatesta et al., 2000]. Even postmitotic neurons can be induced to reenter the cell cycle and generate dividing progenitor cells [Brewer, 1999; Alexanian and Nornes, 2001].

These and other results (summarized in Table I) appear to indicate that cell fate restriction is more apparent than real, and simple culture manipulations may be sufficient to alter any developmental restrictions and the capacity to transdifferentiate may extend to postmitotic cells as well.

HOW CAN ONE EXPLAIN THESE RESULTS?

The multitude of reports on the ability of cells to alter their developmental fates and the equally voluminous data on progressive cell fate restrictions posit two extreme scenarios. Perhaps all cells in the organism are in a state of flux and a stable differentiated phenotype is maintained actively and with difficulty or alternatively, a stable phenotype is normal and altering cell fate is difficult and can only be achieved (perhaps only transiently as well) by complex sequential manipulations. Distinguishing between these extremes has important therapeutic implications as in one case biology has presented us with robust, easily harvested sources of autologous cells for cellular replacement strategies. The ethical dilemma of using fetal tissue and ES cells is neatly bypassed and the issue of immune matching donor tissue has been rendered moot. The alternative scenario that transdifferentiation is not a fate that is simply waiting to happen suggests that biology is less kind and leads to a somewhat less optimistic future for cell therapy.

Unless we identify a reliable source of stem/progenitor cells and figure out how to defeat the bodies immune rejection mechanisms, cell transplantation will remain an unproven possibility. It is, therefore, important to evaluate all reported cases of transdifferentiation to determine whether they represent examples of dedifferentiation or transdifferentiation or whether they can be explained without invoking transdifferentiation as has been suggested in several recent reports. In the subsequent section, we will examine potential explanations for the seeming ability of cells to generate atypical differentiated phenotypes. The various possibilities are summarized in Fig. 3. Overall as will be clear in subsequent sections, in our opinion, normal development requires sequential restriction in developmental potential and these restrictions can be reversed only under special conditions. Some but not all of the transdifferentiation results can be explained by alternative mechanism which we discuss in brief in the next section (Fig. 3).

TAPPING INTO NORMAL DEVELOPMENTAL PATHWAYS

Ectoderm to mesoderm transformation is normally seen in neural crest differentiation. Indeed neural crest cells have been shown to generate muscle, bone, cartilage, melanocytes, fibroblasts, smooth muscle as well as neural components of the peripheral nervous system (PNS) ([Ziller et al., 1983]; reviewed in [Rao, 1999]). The ability of CNS stem cells to generate neural crest may explain the mesodermal differentiation demonstrated with NSC transplants. CNS stem cells may generate neural crest, which in turn can generate smooth muscle cartilage and bone. Both fetal and adult NSCs have been shown to generate neural crest and smooth muscle and cartilage in culture [Mujtaba et al., 1998; Tsai and McKay, 2000].

A similar pathway for differention from mesoderm to ectoderm (or vice versa) may exist in other precursor cells as well. Mesodermal to ectodermal transformation is a normal complement of the development of some organs. In kidney, for example, mesodermal cells undergo an epithelial transformation to generate kidney tubules [Herzlinger, 1995]. Mesodermal stem cells or MSCs have been identified. It is possible that these MSCs can generate all mesodermal derivtaives (including hematopoietic lineages) as well as undergo an ectodermal transformation to generate ectodermal derivatives.

We would suggest that this does not represent true transdifferentiation but rather reveals a previously overlooked pathway of normal differentiation, which can be modulated in culture. A careful comparison of transdifferentiation events with known developmental pathways may be revealing in some reported cases of transdifferentiation.

CONTAMINATING POPULATIONS OF CELLS

An alternative explanation to "true transdifferentiation" is the presence of contaminating populations of cells. Many of the reports on transdifferentiation have used non-purified populations of cells (bone marrow, neurosphere

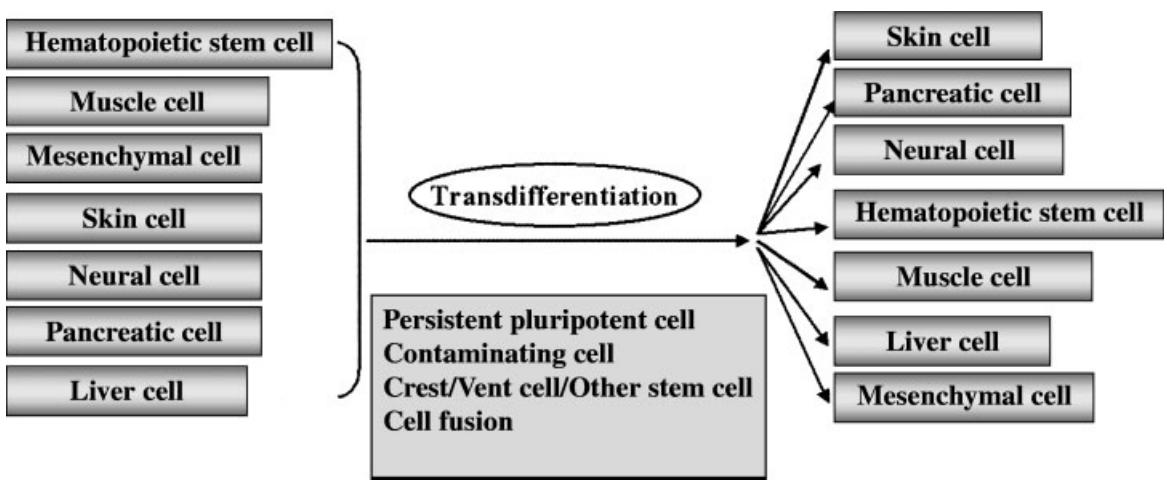

Fig. 3. Atypical differentiation of somatic cells. Atypical differentiation of cells or the generation of unexpected phenotypes can be attributed to several mechanisms and these are summarized.

cultures, etc.) which may contain small populations of cells of a different phenotype. When one dissociates adult tissue, several populations of cells other than parenchymal tissue specific cells are present upon dissociation. Cells identified routinely include endothelial cells from blood vessels, cellular components of blood, connective tissue fibroblasts, Schwann cells from nerve endings as well as the tissue specific cells of interest. In most cases these contaminating populations represent a miniscule number of cells and can be safely ignored. However, when one selects stem cell or precursor cell populations which themselves constitute only a tiny subpopulation, these cells can become a major issue, especially if these cells can proliferate under the same culture conditions as the stem or progenitor cell population. Indeed, fibroblasts are the major contaminating population when Schwann cells are cultured from the peripheral nerve and can beginning from a 1% contamination, become the predominant population after as few as four passages (our unpublished results). Likewise, glial precursors can overrun a dish of neuronal precursors even though they initially represent a tiny contamination (our unpublished results) and NSC cultures can be taken over by faster dividing astrocytes ([Cao et al., 2001] and our unpublished results).

In an interesting series of experiments (using the technique of parabiosis) Weismann and colleagues have shown that hematopoietic stem cells circulate in the blood in small numbers and can be isolated from multiple tissues and organs [Weissman et al., 2001]. The calculated numbers were quite impressive and suggest that when unpurified tissue is dissociated and transplanted, hematopoietic stem cells constitute a significant contaminant and could explain some of the transdifferentiation results reported with non-clonal populations of cells. Indeed, Goodell et al. have suggested that their results are perhaps best explained by the presence of contaminating stem cell populations [McKinney-Freeman et al., 2002].

A second contaminating population that could explain the apparent transdifferentiation into neural tissue are neural crest cells and ventrally emigrating neural tube (VENT) cells. Quiescent neural crest stem cells present in peripheral nerves and organs [Morrison et al., 1999] may generate PNS precursors that subsequently differentiate into neurons, non-neural cells, and myelinating glia. Alternatively, an additional neural derivative, VENT cells, which have also been shown to contribute to cartilage, bone, heart muscle, and hepatocytes [Ali et al., 1999; Sohal et al., 1999a,b,c] during normal development, may be present in non-neural tissue. Markers exist to distinguish contaminating populations and clonal propagation of many cell types is possible. Perhaps only rigorously selected purified populations of cells should be evaluated for their transdifferentiation potential.

PERSISTENCE OF A PRIMITIVE PLURIPOTENT STEM CELL POPULATION

A third possibility that could explain the diversity of cell type differentiation seen with cultured "tissue specific" stem cells is the possibility that a pluripotent population of primitive stem cells persist, in small numbers, in every tissue or organ. These cells could represent the remnants of totipotent cells that are present in early development; investigators may have stumbled upon these cells when testing for transdifferentiation. These residual primitive stem cells could have been set aside at the inner cell mass stage of embryonic development or could represent ectopically localized primordial germ cells. These cells, essentially a contaminant in an isolation, would respond to cues in novel environments to direct their differentiation along an alternate pathway.

Distinguishing between this alternative and true transdifferentiation is difficult unless a detailed clonal analysis with rigorous attention to numbers of such totipotent cells present is performed. Given our relatively limited knowledge of the properties of early stem cells and the factors that regulate their differentiation, this possibility cannot be readily discounted. We note that recent reports have suggested that some clonal populations of stem cells harvested from the adult appear to express markers such as REX and Oct-3/4 which have been considered hallmarks of ES cells [Jiang et al., 2002].

FUSION AND DNA TRANSFER

An additional intriguing possibility that may explain many of the observations of transdifferentiation is the possibility that cells in a mixed culture may undergo spontaneous fusion and the resulting hybrid may demonstrate the abilities of both parent populations. Two recent manuscripts have elegantly demonstrated fusion and gene transfer. Ying et al. [2002] co-cultured

NSCs with pluripotent ES cells and, after selecting for a transgenic marker carried only by the brain cells, recovered stem cell populations. These cells exhibited full pluripotent character, including multilineage contribution to chimaeras. The authors noted, however, that the same population also carried a transgenic marker and chromosomes derived from co-cultured ES cells suggesting that NSCs had not dedifferentiated/transdifferentiated into ES cells, rather they had formed hybrids by cell fusion. They, therefore, proposed that transdetermination consequent to cell fusion could underlie many observations otherwise attributed to an intrinsic plasticity of tissue stem cells. Similar results were reported by Terada et al. [2002], when they co-cultured bone marrow stem cells with ES cells in the presence of IL-3. Like Ying et al., they showed that murine bone marrow cells can fuse spontaneously with ES cells and that spontaneously fused bone marrow cells can acquire the properties of ES cells. Both of these results suggest that cell fusion may be an alternative explanation for the dedifferentiation/transdifferentiation results that have been reported. A detailed genetic analysis will need to be performed to rule out this possibility.

TRUE TRANSDIFFERENTIATION

It may be possible to dismiss some or even many of the reports of transdifferentiation on the basis of the presence of contaminating cells, persistence of primitive cells, cell fusion, gene transfer, or to overenthusiastic interpretation of data. Nevertheless, it is unlikely, in our opinion, that transdifferentiation does not exist at all or cannot be induced. Several examples of unambiguous transdifferentiation can be found and a few examples are detailed below. These results cannot be dismissed easily by invoking alternative explanations.

Early evidence from studies in embryonic development suggests that while there is progressive restriction in developmental potential, this restriction is not absolute and can be modified by altering expression of single genes or altering the environment at specific developmental stages. For example, ablation of the dorsal neural tube transforms cells normally fated to become ectoderm to participate in neural development (see, e.g., [Saldivar et al., 1997]). Myo-D expression in the nervous system can generate skeletal muscle from neural cells (see, e.g., [Boukamp, 1995]) and expression of neural genes in the ectoderm can generate neurons (see, e.g., [Lee et al., 1995]). Perhaps the best examples of such alteration of developmental commitment encoded by combinations of transcription factors have come from homeobox transformations in flies [Johnston and Schubiger, 1996; Campbell and Tomlinson, 1998]. Ectopic expression of vestigial, for example, can convert a *Drosophila* leg to wing [Couso et al., 1995]. Other examples of induced transdifferentiation include expression of C/EBP beta in pancreatic islet cells to convert them to hepatocytes [Rao and Reddy, 1995; Shen et al., 2000] and overexpression of PPAR-gamma in myoblasts to convert them to adipocytes [Hu et al., 1995; Taylor-Jones et al., 2002]. These results cannot be explained except as true transdifferentiation and raise the possibility that changes in single genes may be enough to alter phenotypes in a dramatic fashion.

Equally convincing data has come from somatic nuclear transfer and cell fusion studies. By fusing erythroid cells at different stages of development, Broyles showed that erythroid nuclei of either early or late developmental stage can be reprogrammed and the gene switch can either be reversed in adult erythroid nuclei and/or prematurely-induced in fetal/embryonic erythroid nuclei [Broyles, 1999]. This reprogramming is due to trans-acting factors that are developmental-stage-specific, clearly indicating that progressive restriction during development can be modulated. More recent somatic nuclear transfer experiments have shown that a somatic nucleus from a differentiated cell, when transplanted back into an oocyte, can be reprogrammed to restore totipotency [Wilmut et al., 1997; Wakayama et al., 1998]. While relatively inefficient, the fact that it occurs at all is clear evidence that epigenetic factors control the differentiated state of a cell and that in certain circumstances cells can be induced to alter their differentiation potential. Cloning experiments such as these have been successful in mice, rats, cows, pigs, and sheep suggesting that reprogramming of cell fate depends on environmental signals and can be reversed under suitable conditions in most species.

These and other experiments provide evidence that while the fate of cells is normally restricted to a limited number of possibilities this restriction is not absolute (see [Theise and Krause, 2002] for a detailed discussion) and that

transdifferentiation is possible or can be sometimes induced by altering the expression of a single gene. Cells are likely more plastic at certain stages but clearly, even the most differentiated postmitotic cell, may undergo reprogramming to differentiate into multiple phenotypes including generating an entire embryo. It may be possible to control the process of transdifferentiation provided the mechanisms regulating cell fate restriction are better understood and the factors in cells capable of reprogramming nuclei are better characterized. In the next section, we discuss some of the mechanisms that may regulate cell fate specification and suggest that understanding the mechanisms that regulate cell type specification may provide clues as to whether efficient transdifferentiation into a particular phenotype is possible.

POSSIBLE MECHANISMS BY WHICH RESTRICTION IN CELL FATE MAY OCCUR

The process by which an initially totipotent homogenous cell differentiates to generate distinct daughter stem cells, where the distinctions are heritably stable for the most part has been studied in a variety of cell models. In Figure 4, we summarize major pathways that may regulate cell type specific gene expression. We reason that interference with these pathways may reveal the potential of cells to differentiate into atypical or unexpected fates.

Several results suggest that lineage-specific genes are operative in a totipotent stem cell prior to lineage commitment and strongly support the concept that stem cells express a multilineage transcriptosome. Most genes (including tissue specific genes) are maintained in an open state with low but detectable levels of transcription with higher levels of specific transcription seen in appropriate cell types. Maintenance of an open transcriptosome in multipotent cells likely requires both the presence of positive factors as well as the absence of negative regulators. Factors that maintain an open transcriptosome include as yet unidentified factors such as demethylases, reprogramming molecules present in blastocyst cytoplasm, and regulators of heterochromatin modeling. These positive factors are segregated as early progenitor cells undergo asymmetric cell division. The cell that receives these factors remains undifferentiated while the other daughter either

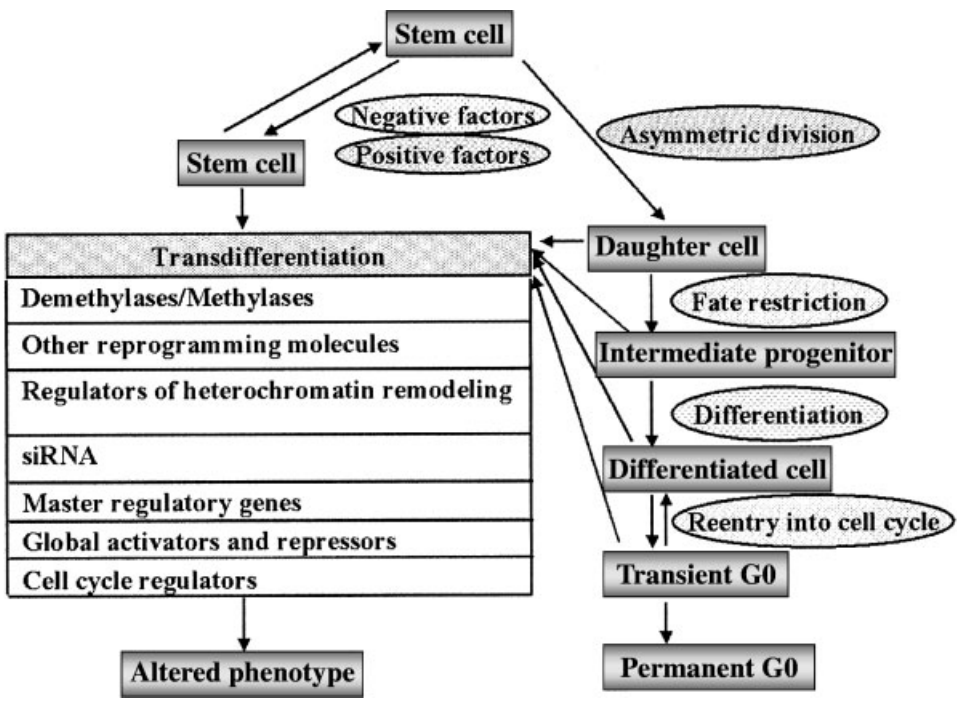

Fig. 4. Mechanisms regulating the maintenance of a stable phenotype. The undifferentiated progenitor cell when undergoing phenotypic differentiation must alter its transciptosome to activate cell type genes and to inactivate inappropriate genes. Both positive and negative regulators have been identified. Note that there are complex interactions between these regulatory pathways that have been omitted for the sake of simplicity.

degrades these factors, or does not receive them to activate cell type specific programs [Knoblich, 1997]. Global activators, global repressors, and master regulatory genes play important regulatory roles in switching on or off cassettes of genes while methylation (reviewed in [Bird and Wolffe, 1999]; [Surani, 2001]), heterochromatin remodeling [Wu and Grunstein, 2000], and perhaps small interfering RNA (siRNA) [Ahlquist, 2002] maintain a stable phenotype by specifically regulating the overall transcriptional status of a cell. Allelic inactivation and genome shuffling further sculpt the overall genome profile to generate sex, organ, and cell type specification. Most adult cells are postmitotic and are held in either transient or permanent (irreversible G0) stage [Sommer and Rao, 2002]. Reentry into mitosis is actively regulated and activation of cell cycle genes leads to apoptosis in cells held in irreversible G0 stage [Sommer and Rao, 2002]. Overall, the establishment and maintenance of the differentiated cell type appears to be tightly regulated by multiple mechanisms that operate at different stages during development. It is important to emphasize that not every mechanism is equally active in all cells and complex interactions occur between the various regulatory molecules.

For transdifferentiation to occur, some or all of these pathways that regulate the differentiation process must be susceptible to extrinsic manipulation. Available evidence from a variety of sources suggests that it is indeed possible to induce transdifferentiation by such manipulations. Schwann cells will transdifferentiate into melanocytes when the neurofibramatiosis gene is mutated [Stocker et al., 1995] and tumors often show evidence of metaplasia or transdifferentiation [Kameyama et al., 2000]. Forced expression of global regulators or treatment with 5-Azacytidine or drugs that modulate heterochromatin remodeling will result in altered differentiation [Robertson and Jones, 2000]. Thus, most of the mechanisms that regulate phenotypic specification are reversible and altering these regulatory mechanisms will permit expression of genes normally never expressed by particular cell types or alter their phenotypic differentiation. However, clearly our understanding of these pathways is limited and we lack precise control of the process. For example, generating DNMT1 (a methylase) null mice has effects on subsets of neurons in the nervous system rather than a global effect one would predict based on its postulated function and expression pattern. Further, the resultant outcome is not transdifferentiation of the cells but cell death, though there is clear upregulation of genes that are regulated by methylation [Fan et al., 2001].

Overall we would suggest that pathways to dedifferentiating or transdifferentiating cells exist and virtually every mechanism that regulates a stable differentiated phenotype can be subverted or modulated in one system or the other. This modulation is sufficient to produce relatively dramatic changes in phenotype although we lack a detailed understanding or a precise control of the process. Framing transdifferentiation as an alteration of the normal regulators of progressive differentiation may allow us to plan more defined experiments and make predictions as to the outcome.

SOME PREDICTIONS

Our discussion of transdifferentiation as an outcome of the alteration in the normal factors that regulate progressive cell fate restriction leads to some specific predictions. We welcome guidance from readers to additional examples and comments as to the overall validity of these predictions.

A general prediction that we believe holds true is that transdifferentiation or altering the fate of fetal cells is likely to be easier than altering adult stem cell fates or the fates of postmitotic cells. We also believe that transdifferentiation within a germ layer or within the tissue may be easier and involve fewer steps than transdifferentiation across germ layers. We, therefore, predict that it will be easier to generate neurons from astrocytes or liver from pancreas rather than intestinal cells from neuroepithelium.

It is unlikely that all regulatory pathways to inhibit transdifferentiation are active in all cells at all stages. Rather, different pathways to transdifferentiation exist in different cell types and different strategies to induce alterations in phenotype will be required. This prediction offers both a promise and a challenge. It is possible that we may identify a mechanism that is relatively specific in inducing a particular type of required transdifferentiation. The challenge will be finding the specific factor or combination of factors.

We suggest that transdifferentiation is normally tightly regulated as uncontrolled or ectopic, inappropriate differentiation is likely to be harmful in most conditions. Therefore, it is unlikely that we can subvert this normal tight regulation with precision and permanence with simple culture manipulations. Rather, more complex manipulations will be required. Further, even if we successfully transdifferentiate cells, this change may not be heritable in subsequent generations and daughter cells may revert to their original phenotype. We further suggest that results obtained with factors that regulate transdifferentiation in one species will not be readily extrapolated to other species as intrinsic properties and mechanisms regulating gene expression differ (note, e.g., telomerase biology is quite different between rodents and humans).

Examining the process of transdifferentiation as aberrant fate restriction suggests some therapeutic strategies to achieve controlled transdifferentiation. Identifying the cytoplasmic factors that regulate nuclear reprogramming may identify important global regulators of fate restriction. Molecules regulating methylation and heterochromatin remodeling are likely to be useful candidate molecules to regulate transdifferentiation. Equally useful we predict will be siRNAs, where we may be able to co-opt a natural regulatory process to selectively derepress or activate specific subsets of genes. Finally, we would suggest that if forced senescence is a commonly used mechanism to prevent reentry into the cell cycle and to inhibit dedifferentiation or transdifferentiation, then apoptosis inhibitors may be useful both for understanding and manipulating the process of transdifferentiation.

SUMMARY AND CONCLUSION

Any transdifferentiation event needs to be carefully analyzed to distinguish "true transdifferentiation" from other alternative explanations. True transdifferentiation may be best understood in a framework of a modulation of factors that normally regulate progressive cell differentiation. These regulators include methylation/demethylationheterochromatin remodeling, global activators, and repressors, etc. Altering these regulators in precise and controlled ways that will be specific for cell types and stages of development may allow one to alter cell fate in specific subsets of cells. In the absence of documented controlled transdifferentiation (even when it exists) and the potential issues that remain to be addressed, we would suggest that transdifferentiation remains an exciting but unproven alternative for cellular therapy.

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