# HOW CELLS CHANGE THEIR PHENOTYPE

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Recent attention has focused on the remarkable ability of adult stem cells to produce differentiated cells from embryologically unrelated tissues. This phenomenon is an example of metaplasia and shows that embryological commitments can be reversed or erased under certain circumstances. In some cases, even fully differentiated cells can change their phenotype (transdifferentiation). This review examines recently discovered cases of metaplasia, and speculates on the potential molecular and cellular mechanisms that underlie the switches, and their significance to developmental biology and medicine.

METAPLASIA
The conversion of one cell or tissue type into another. Includes transdifferentiation and also conversion between undifferentiated stem cells of different tissues.

STEM CELL
A cell that has the potential to divide and produce a replica cell as well as differentiated progeny.

DIFFERENTIATION
The synthesis of proteins that are produced selectively in a single cell type (for example, albumin in hepatocytes). Differentiation is generally reflected in specialized structure (such as bile canaliculi) and function (such as synthesis of bile).

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METAPLASIA — the conversion of one cell or tissue type into another — is an important phenomenon for three reasons[1]. First, understanding the molecular basis of tissue-type switching is likely to increase our knowledge of normal developmental mechanisms. Second, some metaplasias are precursors to cancer, or are important in human pathology. Third, and perhaps of most interest at present, understanding the rules for tissue-type switching should improve our ability to reprogramme STEM CELLS for the purpose of therapeutic transplantation.

The subset of metaplasias that is known as transdifferentiations is particularly remarkable. Transdifferentiation is the conversion of one differentiated cell type to another, with or without an intervening cell division, so it challenges our preconceived ideas about the nature of the differentiated state. It used to be generally accepted that the terminal differentiated state is fixed, but it is now clear that DIFFERENTIATION can sometimes be reversed or altered. This review examines recent examples showing both metaplasia of stem cells, characteristic of one embryonic germ layer giving rise to cells from another germ layer, and also transdifferentiation between fully differentiated cell types. This is a particularly timely moment to review the field because of the remarkable array of transformations that have been uncovered in recent work on the transplantation of adult stem cells.

## Criteria for metaplasia

In general, naturally occurring metaplasias (BOX 1) are associated with excessive growth that arises through either wound healing or an abnormal response to hormonal stimulation. Whatever the underlying mechanism — whether somatic mutation of key developmental genes, or EPIGENETIC CHANGES in their expression — excessive growth increases the probability that a macroscopic lesion will be formed from a microscopic one, which could initially be as small as a single transformed cell.

Not all metaplasias represent transformations to tissues that are themselves normal, and many of the final states are atypical or DYSPLASTIC. However, the cases of switching between normal tissue types are those of most biological interest. They are the vertebrate counterparts of HOMEOTIC MUTATIONS in insects[2,3], or of the epigenetic switching of *Drosophila melanogaster* IMAGINAL-DISC types during long-term culture, which is called 'transdetermination'[4].

Although the pathological literature is rich in examples of metaplasia, these examples are usually documented from the histology of static preserved specimens. This means it can be difficult to rule out the possibility that the ectopic tissue has arisen by cell migration rather than by metaplasia. By contrast, in experimental biology it is possible to prove that metaplasia has occurred. To do this, the differentiation or DETERMINATION state of the starting and final cells must be well characterized by morphological and/or molecular

**Box 1 | Naturally occurring metaplasias**

**The literature of human pathology is filled with examples of metaplasia[56–58]. For example, ectopic bone formation is quite common in surgical scars, muscle that is subjected to repeated trauma, or the walls of sclerotic arteries. The epithelia of the respiratory tract or urinary bladder can undergo squamous metaplasia, a precursor to squamous cell carcinoma.**

**Foci of ectopic glandular epithelium can also occur, particularly in the alimentary canal. For example, intestinal metaplasia of the stomach can generate patches of intestinal crypts and villi within the stomach. Barrett's metaplasia of the oesophagus can develop as a result of duodenal–oesophageal reflux[59,60] and is considered the precursor lesion for development of oesophageal adenocarcinoma. In the female reproductive tract, there are also numerous examples of patches of ectopic epithelium; for example, patches of tubal or endocervical epithelium are not uncommon within the endometrial lining of the uterus.**

**Although examples of metaplasia are common in human pathology, this does not mean that all metaplasias are necessarily pathological processes.**

criteria, and there must also be some means of unambiguously tracing the lineage from starting to final cells. In tissue-culture systems, this can sometimes be done by direct observation, whereas *in vivo* a genetic marker is required. These prerequisites were originally outlined by Eguchi and Kodama[5] as conditions for proving the occurrence of transdifferentiation, and the occurrence of transdifferentiation in regeneration has been recently discussed by Brockes and colleagues[6].

Genetic markers have been widely used in recent studies of transplantation of stem cells. Popular choices are the ROSA26 mouse, which shows ubiquitous expression of *Escherichia coli* β-galactosidase[7]; transgenic green fluorescent protein (GFP) *in vitro* or *in vivo*[8–10]; or, for animal and human studies, the presence of the Y chromosome in grafts from male to female[11,12] (BOX 2). In future, it is likely that the CRE/*lox* technique will be applied to monitor metaplasia *in vivo*, as it can be used to irreversibly label a lineage after transient activation of a tissue-specific promoter[13]. However, there are caveats to using this technique; interpretation of the experiments depends heavily on the stringency or leakiness of the promoters that are used.

Metaplasias normally involve cell division, but there are a few cases of direct transdifferentiation without intervening cell division[1,14]. These can be documented by direct observation *in vitro*, or by non-uptake of 5′ BROMODEOXYURIDINE (BrdU) over the period of transformation. Like Eguchi and Kodama[5], Beresford[14] has also proposed several stringent criteria to substantiate whether a process really occurs by direct transdifferentiation. These are: that the cells undergoing transdifferentiation show a mature, stable phenotype; that the cells change their phenotype; and that the cells do not divide before altering their differentiated state.

## Metaplasia of stem cells

A stem cell is an undifferentiated cell that can divide indefinitely and that produces progeny, which include both more stem cells and cells that are destined to become differentiated cells[15] (FIG. 1). In many tissues, several differentiated cells can be formed by a single stem cell. Stem cells have been isolated from many sources including the blood[16], skin[17,18], central nervous system (CNS)[19], liver[20,21], gastro-intestinal tract[22] and skeletal muscle[23].

Although there is little direct evidence, it is attractive to speculate that the characteristics of stem cells of a particular tissue resemble those of the embryonic rudiment for that tissue. It used to be presumed that stem cells were determined (that is, irreversibly COMMITTED) to form the cell types of that tissue alone. Now it is not so simple. In fact, Helen Blau and colleagues[24] have recently argued that stem cells are complex creatures with an ability to show plastic behaviour depending on the environment they find themselves in, including, in particular, the signals from damaged tissues.

The first examples to show that stem cells from one tissue could generate cells from an unrelated tissue came from transplantation of bone marrow in both animals and humans. Mavilio, Cossu and colleagues[25] showed the formation of muscle from transplanted bone marrow that was taken from a transgenic mouse line in which a muscle promoter (the myosin light chain 3F promoter) was used to drive expression of nuclear β-galactosidase. The authors isolated bone-marrow cells from these transgenic animals and injected them into an immunodeficient mouse strain in which myonecrosis was induced chemically with cardiotoxin. In the marrow cells themselves, there was no expression of β-galactosidase, but this was activated in cells that became associated with the muscle of the host. Although this transdifferentiation occurred, it was much slower (weeks) compared with injection of the resident myogenic precursor (satellite) cells, which differentiated to muscle much more quickly (days). This time difference indicates that metaplasia from bone marrow to muscle might be a multistep process, which involves migration, commitment and eventually terminal differentiation.

There are now many examples of bone-marrow-derived stem cells that contribute to various differentiated cell types after grafting, and some of these are listed in TABLE 1. Most studies so far have used unfractionated bone marrow as a source of cells, but bone marrow contains two well-characterized types of stem cell. The haematopoietic stem cells normally give rise to the cells of the blood and immune system, whereas the mesenchymal stem cells normally form CHONDROCYTES and OSTEOBLASTS. An important question is: which stem cell produces which differentiated cell type in the grafting experiments?

***Bone marrow to hepatic epithelium.*** Several studies have shown the potential for bone-marrow cells to produce cells of the liver[11,12,26–28]. These have all used lineage markers to verify the origin of the donor cells. They show that the donor bone-marrow cells can migrate and deposit themselves in the liver, and can produce both cells of the bile ducts and true hepatocytes.

Some of the best evidence for bone-marrow cells giving rise to differentiated liver-cell types comes from Grompe and co-workers[28]. The authors tested the therapeutic potential of bone-marrow-derived haematopoietic stem cells in a mouse model for HEREDITARY TYROSINAEMIA TYPE I,

EPIGENETIC CHANGE
A stable change in phenotype that arises from processes other than the alteration of sequences of bases in genomic DNA.

DYSPLASIA
A cellular growth abnormality in which the cellular appearance is altered and tissue architecture might be disturbed.

HOMEOTIC MUTATIONS
A class of mutations in which a given tissue or body part develops in the same way as one that is normally present in another part of the body.

IMAGINAL DISCS
Single-cell layer epithelial structures of the *Drosophila* larva that give rise to wings, legs and other appendages.

DETERMINATION
The irreversible commitment of a cell to follow a pathway of development.

CRE/*lox*
A site-specific recombination system derived from *Escherichia coli* bacteriophage P1. Two short DNA sequences (*lox* sites) are engineered to flank the target DNA. Activation of the Cre-recombinase enzyme catalyses recombination between the *lox* sites, leading to excision of the intervening sequence.

5′ BROMODEOXYURIDINE
A base analogue of thymidine, which is often used experimentally to label dividing cells.

COMMITMENT
The intrinsic nature of a cell to follow a pathway of development.

CHONDROCYTE
A differentiated cell of cartilage tissue.

OSTEOBLAST
A mesenchymal cell with the capacity to differentiate into bone tissue.

HEREDITARY TYROSINAEMIA TYPE I
An autosomal recessive condition caused by mutation of the *FAH* gene. Patients have increased levels of tyrosine in the blood, and symptoms include deterioration of the liver and accumulation of other amino acids, such as methionine in the blood and urine.

PARENCHYMAL TISSUE
All tissue other than lymphoid tissue. Lymphoid tissue is derived from the bone marrow, whereas parenchymal tissue is not.

ASTROCYTE
A star-shaped glial cell that supports the tissue of the central nervous system.

OLIGODENDROCYTE
A glial cell in the nervous system that forms a myelin sheath around axons.

MICROGLIA
Phagocytic immune cells in the brain that engulf and remove cells that have undergone apoptosis.

**Box 2 | Common methods for lineage tracing in studies of metaplasia and transdifferentiation**

- **The ROSA26 mouse, which shows ubiquitous expression of *Escherichia coli* β-galactosidase. Grafts of ROSA26 tissue to unlabelled mice retain β-galactosidase expression, which can be detected using the X-gal histochemical reaction.**
- **Transgenic green fluorescent protein (GFP) *in vitro* and *in vivo* under ubiquitous promoters or, occasionally, a tissue-specific promoter. Grafts of GFP tissue retain fluorescence. In addition, because the GFP protein is quite stable, cells retain fluorescence for a few days, even after a promoter that drives the *GFP* gene is turned off.**
- **The presence of the Y chromosome in grafts from male to female. This can be detected by *in situ* hybridization for a Y-specific DNA sequence.**
- **The Cre/*lox* technique (FIG. 4), which enables permanent activation of a reporter gene after transient activation of a tissue-specific promoter.**

the *FAH*$^{-}$ mouse, which lacks the gene that encodes fumaryl-acetoacetate hydrolase (FAH)[29]. This mutant mouse model can normally survive only if treated with the drug NBTC (2-(2-nitro-4-trifluro-methylbenozyl)-1,3-cyclohexanedione), which prevents liver failure induced by tyrosinaemia by inhibiting an enzyme upstream of FAH. Following a rigorous method for purification of haematopoietic stem cells, the donor cells (from the ROSA26 line that carries a ubiquitously expressed *E. coli lacZ* gene) were transplanted into the mouse model of tyrosinaemia type I. The haematopoietic stem cells were injected into lethally irradiated adult hosts to ensure rapid engraftment, and produced hepatocytes *in vivo*.

This study conclusively shows that haematopoietic stem cells have the potential to give rise to hepatocytes, a transformation that transgresses the traditional specificity of germ layers and shows the therapeutic potential of the technique. Also, in a recent striking study, Krause *et al.*[30] showed that a single haematopoietic stem cell (based on the principle of limiting dilution) could differentiate into the epithelial cells of the liver as well as lung, gastrointestinal tract and the skin. Other evidence indicates that haematopoietic stem cells can contribute to the cells of the renal PARENCHYMA[31].

***Bone marrow to brain cells.*** The predominant cell types of the CNS are neurons, ASTROCYTES, OLIGODENDROCYTES and MICROGLIA. The first three are of ECTODERMAL origin whereas microglia are considered to be a specialized type of macrophage, normally derived from the haematopoietic stem cells.

One of the first studies to show that haematopoietic progenitor cells (retrovirally marked or using the Y chromosome as a marker; BOX 2) that are derived from bone marrow could generate astrocytes of the CNS came from Eglitis and Mezey[32]. These authors presented evidence based on the expression of glial fibrillary acidic protein (GFAP), an intermediate filament protein found in astrocytes. In another study, Priller *et al.*[10] transferred the *GFP* gene into donor bone-marrow cells and then identified GFP-expressing cells in mice that received the transplant after lethal irradiation[9]. Initially, GFP-positive cells were found in the circulation after replenishment of the haematopoietic system, which then invaded the brain. In the cerebellum, donor-derived PURKINJE CELLS were not seen 4 months after transplantation, but were present after 12 months and then survived for at least another 3 months.

On the basis of the characteristic morphology of these cells and their expression of glutamic acid decarboxylase (the enzyme responsible for synthesis of the neurotransmitter GABA) and calbindin-D28K, the authors showed that donor-derived neurons appeared morphologically normal and functional, and continued to survive as the mouse aged[9]. The authors explained the initial lack of colonization as being due to the time it takes for the natural death of Purkinje cells. If this is true then it means that bone-marrow-derived stem cells can preferentially regenerate neurons that are normally lost to ageing. One question raised from this and similar studies is what are the signalling molecules that induce transdifferentiation? Whether this is a local factor that is released in the microenvironment of the damaged cells or a more systemic factor remains to be determined.

These examples show that haematopoietic stem cells — which are considered tissue-specific stem cells of mesodermal origin — are capable of metaplasia to both ectodermal and ENDODERMAL cell types. Some authors have considered this as evidence for complete PLURIPOTENCY of the haematopoietic stem cells, or even of a continuous cell turnover such that haematopoietic stem cells provide cells for regeneration of all other tissues throughout life. Although both are possibilities, the existing evidence does not prove the case. The metaplasia probably initially occurs in only small numbers of cells that are surrounded

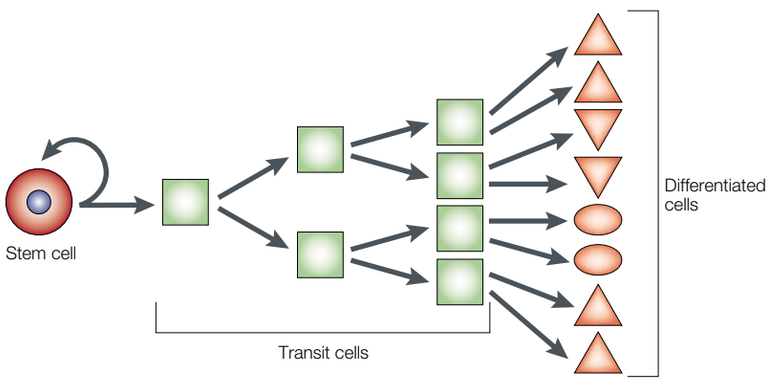


Figure 1 | **Stem cells can undergo self-renewal and generate transit cells and, finally, differentiated cells.** Stem cells are defined as those cells that are undifferentiated and can divide to produce further stem cells, as well as cells that are destined to become differentiated cells. In most cases, stem cells can produce more than one type of differentiated progeny (multi- or totipotent). Adapted with permission from REF. 15 © (2001) Blackwell Science Ltd.

Table 1 | **Stem cells undergo metaplasia to various tissues**

| Progenitor | Derivative | References |
|---|---|---|
| Bone-marrow-derived cells | Skeletal muscle | 25, 61 |
| | Hepatocytes | 11, 12, 25–28, 30 |
| | Endothelial cells | 8, 62, 63 |
| | Purkinje neurons | 9, 64, 65 |
| | Cardiac muscle | 61, 63, 66 |
| Muscle | Bone marrow | 34–36 |
| Oligodendrocyte | Neurons | 33 |
| Neural | Blood cells | 67 |
| | Muscle | 68 |
| | Multiple embryonic tissues | 69 |

ECTODERM
The outermost germ layer of the developing embryo. It gives rise to the epidermis and the nerves.

PURKINJE CELLS
Large, pear-shaped cells of the cerebellum, which are connected to multi-branched nerve cells that cross the cerebellar cortex.

ENDODERM
The innermost germ layer of the developing embryo. It gives rise to the epithelia of the lung, digestive tract, bladder and urethra.

PLURIPOTENCY
A pluripotent stem cell can give rise to more than one differentiated cell type.

by the host tissue. The example of mass tissue colonization in the *FAH*$^-$ mouse arises because there is selection for *FAH*$^+$ cells over a prolonged period.

As far as the mesenchymal stem cells are concerned, Verfaillie and colleagues[8] have studied their potentialities after long-term *in vitro* culture of a population that they refer to as mesenchymal progenitor cells. It was possible to induce osteoblasts, chondrocytes, myocytes, adipocytes or endothelial cells using appropriate culture conditions. Mesenchymal stem cells are generally thought to be the natural progenitors for cartilage and bone, so the formation of several other cell types is an indication of metaplasia.

***Other examples.*** Toma *et al.*[18] have recently described the identification and isolation of a new type of stem cell that was derived from the dermis of the skin. These stem cells were termed skin-derived precursor (SKP) cells. The SKP cells could be induced to differentiate by culturing on poly-lysine and varying the concentration of serum in the culture medium. In the absence of serum they differentiate into neurons and GLIAL CELLS; with the addition of 3% serum they differentiate into smooth-muscle cells; and increasing the serum to 10% causes the SKP cells to differentiate into adipocytes. Because small dermal biopsies are easy to do, this is an interesting potential source of stem cells for therapeutic transplantation.

Oligodendrocyte precursors are progenitors from the optic nerve, and were thought to be already committed to form glial cells — either type-2 astrocytes or oligodendrocytes. In a recent study, Kondo and Raff[33] caused oligodendrocyte precursor cells to become astrocytes by culture with fetal calf serum or bone morphogenetic proteins. They then treated them with fibroblast growth factor (FGF)-2, which induced the cells to convert to neurons. This indicates a reversal of developmental commitment back to a neuroepithelial-type cell that can form both neurons and glia.

The transdifferentiation from muscle to blood has also been proposed by different labs[34–36]. These labs investigated the possibility that cells that are derived from mouse skeletal muscle could generate cells of the haematopoietic lineage. For example, Goodell and colleagues[34] transplanted a crude mixture of pre-cultured skeletal muscle cells and observed the generation of myeloid, T and B cells after lethal body irradiation in a competitive transplantation assay. Gussoni *et al.*[35] also used fluorescence-activated cell sorting (FACS) to isolate a population of cells from muscle that were similar to haematopoietic stem cells. When transplanted into lethally irradiated mice, the haematopoietic system was reconstituted. It is not known whether the haematopoietic cells were derived from the myogenic stem-cell population (the satellite cells), or whether they are derived from another cell type with stem-cell characteristics that is resident in muscle.

## Transdifferentiation of differentiated cell types

***Pancreas to liver.*** The appearance of hepatocytes in the pancreas is one well-documented example of transdifferentiation. Hepatocytes appear in the pancreas of hamsters or rats in response to various experimental treatments — for example, treatment of rats with a copper-deficient diet[37–40], or after transplantation of epithelial cells[41], and in transgenic mice that overexpress keratinocyte growth factor in the pancreatic islets[42]. The reverse transdifferentiation (liver to pancreatic exocrine cell) is induced in the livers of rats after treatment with polychlorinated biphenyls[43].

This ability to interconvert presumably reflects the close developmental relationship between the pancreas and liver: they both arise from the same region of the endoderm. Recently, we have produced two *in vitro* models for the transdifferentiation of pancreatic cells to hepatocytes[44]. The models involve treatment of either the pancreatic cell line AR42J-B13 or embryonic pancreatic buds with the synthetic glucocorticoid dexamethasone and oncostatin M (a member of the interleukin (IL)-6 family). The transdifferentiated cells express a range of liver proteins, including transferrin, transthyretin, albumin and glucose-6-phosphatase, and arise, at least in part, from pancreatic exocrine cells.

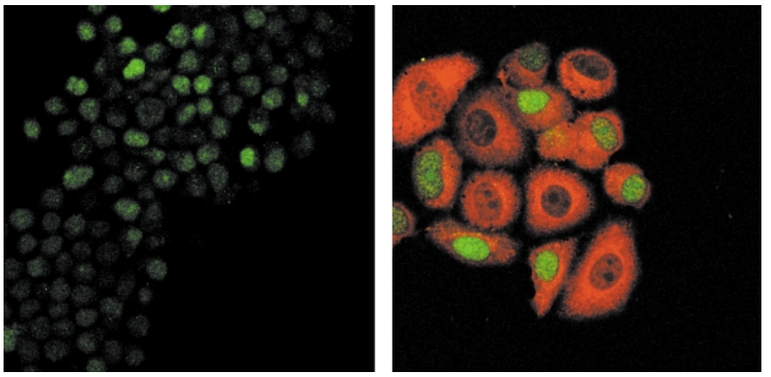

Figure 2 | **Transdifferentiation of hepatocytes from pancreatic exocrine cells.** The pancreatic cell line AR42J-B13 is shown, and contains green fluorescent protein (GFP) under the control of the pancreatic exocrine elastase promoter. The nuclei of the cells are positive because the GFP contains a nuclear-localization signal. After treatment with the glucocorticoid dexamethasone, the cells that have undergone transdifferentiation lose activation of the promoter, but the GFP protein is more stable and persists in the cell for several days. Some cells that were treated with dexamethasone contain both GFP and a liver marker (glucose-6-phosphatase), which indicates that these nascent hepatocytes must, at one time, have had an active elastase promoter and were therefore differentiated exocrine cells. Adapted with permission from *Nature Cell Biology* (REF. 44) © (2000) Macmillan Magazines Ltd.

This ancestor–descendant relationship was shown through the stability of GFP. Using the elastase promoter to drive GFP, we showed that, in transdifferentiating cells, both a liver marker and GFP were present, which indicates that the nascent hepatocytes must once have been differentiated exocrine cells (FIG. 2). In agreement with this observation, it was previously shown that rat hepatocytes arise from exocrine cells after treatment with the hypolipidaemic compound, ciprofibrate[45].

We have also identified the molecular basis of the switch, which involves induction of the transcription factor CAAT/enhancer-binding protein (C/EBP)-β. Transfection of *C/EBP*β into AR42J-B13 cells provokes transdifferentiation, whereas introduction of the dominant-negative form of C/EBPβ (liver-inhibitory protein) prevents the transdifferentiation. Further evidence for a role for C/EBPβ in liver development is indicated by the fact that this factor is bound to the albumin enhancer in embryonic hepatocytes at embryonic day 12.5 (REF. 46). The function of C/EBPβ (and the C/EBP family in general) in liver development awaits further clarification as the family shows a certain degree of redundancy.

***Myoblasts to adipocytes.*** G8 myoblasts are a tissue-culture model for myogenesis and can spontaneously differentiate into myotubes when cultured in medium that contains fetal calf serum. Myoblasts are generally considered fully committed to muscle differentiation, as they express muscle-specific transcription factors (MyoD, myogenin, MRF4, Myf-5). However, these cells can be caused to transdifferentiate to adipocytes after introduction of the transcription factors C/EBPα and peroxisome-proliferator-activated receptor (PPAR)-γ, and culture in a hormone cocktail consisting of dexamethasone, a synthetic leukotriene and insulin[47]. Another myoblast cell line, C2C12, was converted to adipocytes after transfection with a dominant-negative version of the transcription factor TCF4 (REF. 48). The cells developed lipid droplets and expressed adipocyte fatty acid binding protein. TCF4 is likely to be a target of the Wnt signalling pathway, and the authors propose that Wnt-10b is probably the factor that is involved in the endogenous regulation of adipogenesis[48].

***Wolffian regeneration.*** Perhaps one of the most studied examples of transdifferentiation is the regenerating lens in amphibia, although this also occurs in other animals, including the chick[5]. In the process known as Wolffian regeneration, a new lens is formed from the iris. During normal development, the lens is formed from the epidermis, whereas the iris is formed from the optic cup, which is derived from neuroepithelium. When the lens is surgically removed, the pigmented cells of the iris become depigmented and proliferate to produce a new lens, which is morphologically indistinguishable from the lens that is removed surgically.

Eguchi and Kodama[49] have elegantly shown the potential use of tissue-culture models for studying this example of transdifferentiation *in vitro*. Using iris pigment epithelial cells isolated from 1-day-old chicks, the authors first induced dedifferentiation by culture with EdFPH medium, which is a cocktail that contains Eagle's MEM, dialysed fetal bovine serum, phenylthiourea and crude hyaluronidase[48]. The cells then lost the pigment granules containing melanin, proliferated intensely and de-differentiated. After this, the cells were cultured with ascorbic acid, which induced the appearance of lentoids (small clusters of lens cells) and was accompanied by the upregulation of *Pax-6*, a transcription factor implicated in development of the eye[50]. Both *Pax-6* and another homeobox-containing gene, *Six-3* (the mouse homologue of the *Drosophila sine oculis* gene), have been found to induce ectopic lens development in fish[51] and *Xenopus laevis*[52].

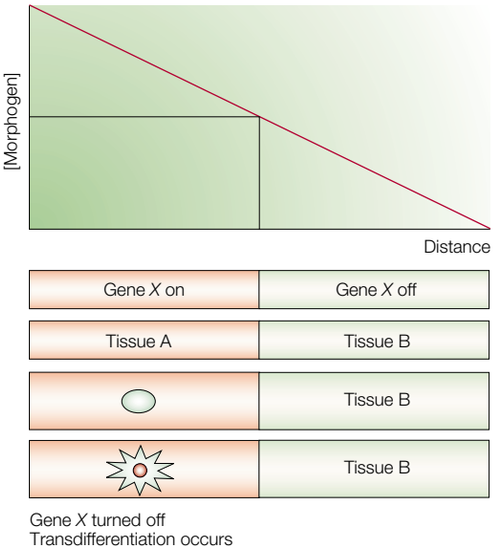


Figure 3 | **A theoretical model for the occurrence of transdifferentiation.** During embryonic life, the rudiments arise for two tissues termed A and B. These neighbouring tissues arise by the activation of a transcription factor X in part of the cell sheet. Assuming that the transcription factor X continues to distinguish the tissue-specific stem cells postnatally, then a change to the microenvironment or a somatic mutation could alter the expression of X and will induce ectopic foci of the neighbouring tissue.

## The cellular and molecular basis of metaplasia

***Cell biology.*** The usual framework for thinking about metaplasia is indicated by the model in FIG. 3. In normal development, tissues A and B are presumed to arise from a single-cell sheet. They are formed because a morphogen gradient induces activation of the gene *X* in part of the region and causes it to become SPECIFIED to form the tissue A. (X is presumed to be a transcription factor that regulates many genes.) The uninduced region follows the default pathway to become tissue B.

If stem cells are similar to cells of the embryonic rudiment for the tissue in question, they will show expression of *X* in tissue A but not in tissue B. So if, at some future time, *X* is inactivated in one or more cells of A,

GLIAL CELLS
Non-neuronal cells of the central nervous system, which comprise astrocytes, oligodendrocytes, microglia and ependymal cells.

SPECIFICATION
A labile form of commitment, which can be altered by environmental signals.

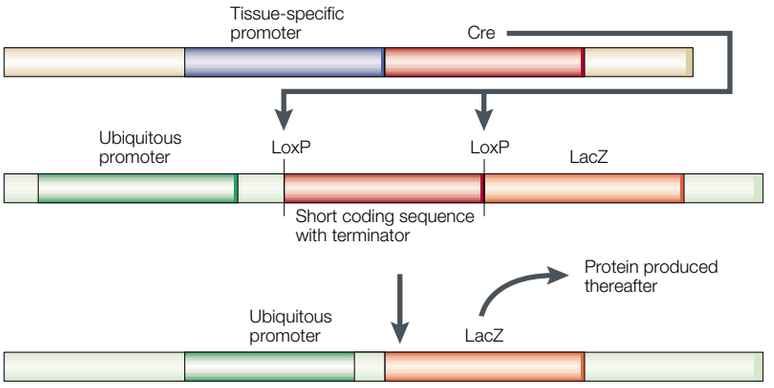


Figure 4 | **The Cre/*lox* system can be used for lineage analysis.** Cre is a recombinase enzyme from the bacteriophage P1, which can excise segments of DNA that are flanked by binding sequences termed Lox*P* sites. A tissue-specific promoter can be used to drive the Cre, which can then excise an inhibitory segment of DNA and activate a reporter such as LacZ or green fluorescent protein (GFP). In this way, the descendant cells of those in which the promoter was originally turned on will continue to express β-galactosidase or GFP, irrespective of whether the cells later express a different phenotype. Adapted with permission from REF. 15 © (2001) Blackwell Science Ltd.

this will produce a focus of metaplasia to tissue B. In the presence of tissue damage and regeneration, the focus might grow to a size where it becomes visible. The evidence for this model comes mostly from the naturally occurring metaplasias (BOX 1). In general, they occur between tissues the rudiments of which are neighbours at the time of formation in the embryo, so are likely to be distinguished by the expression of just one or a few genes. Furthermore, they are nearly always associated with situations of tissue regeneration.

At a cellular level, the main question is whether metaplasias are initiated by somatic mutation (for example, a loss-of-function mutation of *X*) or by environmental stimuli (for example, a signal turning *X* off). It is difficult to know what is happening to cells in the bone-marrow grafting experiments. But with *in vitro* systems, such as the pancreas-to-liver model, it is clear that numerous cells are transformed simultaneously, so this must be due to an environmental signal rather than somatic mutation.

The next question is whether metaplasia occurs during normal life; for example, whether haematopoietic stem cells of the bone marrow normally populate other tissues in a slow and continuous manner. This is not known, and to find out we will need to mark marrow stem cells genetically, without carrying out a graft, which is usually associated with severe doses of radiation that provoke tissue damage as well as death of the host bone marrow. The Cre/*lox* system offers a potential means for doing this as the Cre recombinase can be driven by a tissue-specific promoter and used to activate a reporter gene by excision of an inhibitory DNA sequence (FIG. 4). This will then label all descendant cells of those that had the active Cre promoter, regardless of their subsequent state of differentiation[13].

Some of the transformations described above seem to violate our normal understanding of development because they cause changes across germ layers. It is generally felt that the ectoderm, MESODERM and endoderm are not only morphological layers of cells, but also correspond, to some extent, to the earliest step of commitment during development. A conversion, for example, from haematopoietic stem cells to liver or neurons represents an erasure of this commitment. However, the transplantation results are often from a very small number of cells; and cases in which large-scale organ replacement has occurred involve long growth periods, under selection, starting from a very small number of graft cells[28]. Marrow grafting could be done directly into the target tissue or it could be intravenous, but, in either case, individual cells ending up in the liver or brain will be surrounded by host cells. Embryologists have always known that isolated cells are more labile than cells that are surrounded by others of their own kind, so it is perhaps not surprising that grafted stem cells should often populate unexpected tissues. This behaviour is not incompatible with the idea that the stem cells, in their normal *in vivo* environments, retain the developmental commitment of the embryonic rudiment that gave rise to them.

***Molecular biology.*** At a molecular level, metaplasia must arise from the change in expression level of key developmental genes. These genes are among the homeotic or 'master switch' genes that determine which part of the body is formed by each region of the embryo. In normal development, particular combinations of these genes are activated in each region through the action of inducing signals. Each combination encodes a particular state of developmental commitment. The protein products of the homeotic genes are transcription factors, and their function is to regulate the next level of genes in the hierarchy, which eventually leads to individual tissue types.

Most of the identification of the genes that underlie metaplasia has been made using *in vitro* systems; for example, the role of C/EBPβ in converting pancreas to liver, and of C/EBPα and PPAR-γ in converting myoblasts to adipocytes. An interesting *in vivo* example, which recalls the naturally occurring metaplasia of the gut, arises in the knockout mouse for the *Cdx2* gene[53]. Cdx2 is a transcription factor, normally expressed in the posterior gut endoderm. The knockout is an early lethal because Cdx2 is essential for the function of the TROPHOBLAST. But heterozygotes are viable and have polyp-like lesions in the intestine, which contain areas of keratinized, stratified squamous epithelium that resemble oesophageal epithelium[53]. Although the lesions do not arise by loss of the *cdx2*$^+$-bearing chromosome, they do show suppression of Cdx2 expression from the good allele, so represent the results of local inactivation of this homeotic gene. Remarkably, the oesophagus-like lesions are flanked by HETEROTOPIC gastric and small intestinal epithelium, which indicate that INTERCALARY REGENERATION has occurred, and is provoked by the disparity of developmental commitment between the focus of oesophagus and the surrounding colonic tissue.

MESODERM
The middle germ layer of the developing embryo. It gives rise to the musculoskeletal, vascular and urinogenital systems, and to connective tissue (including that of the dermis).

TROPHOBLAST
The surface cell layers of an embryo at the blastocyst stage.

HETEROTOPIAS
A misplaced tissue, which arises during embryogenesis.

INTERCALARY REGENERATION
Regeneration that occurs at a junction between experimentally joined body parts, which results in the re-formation of parts that normally lie between them.

In contrast to the stem-cell metaplasias, many transdifferentiation events occur between cell types that are closely related in embryonic origin, so might be distinguished by the state of just one or a few genes. In such cases, it is easy to see that the change in expression of one gene might be enough to convert one cell type to another. However, even if metaplasia leads to normal cell types, it does not follow that the configurations of homeotic gene activity that produce them are normal ones. We know that in experimental situations, certain genes can drive various cell types to a particular tissue type; for example, *MyoD* will convert many cell lines to muscle[54] and *pax-6* (REF. 55) will convert many *Drosophila* imaginal discs to eye tissue. It could therefore be possible to find genes that will drive, for example, haematopoietic stem cells to liver in a single step, even though in normal development these cell types are many steps apart in terms of developmental commitment events.

## Conclusions and the future

It is now apparent that transdifferentiations and other metaplasias are a biological reality. Whether they really do occur on a day-to-day basis during regeneration after normal physiological damage has yet to be established. Cre/*lox* experiments will be needed to establish the physiological significance of these observations, which at present rely heavily on inducing damage through total body irradiation.

Although some examples of metaplasia and transdifferentiation have been shown to occur *in vivo*, many experiments have been done *in vitro*, and it is not clear whether these changes in phenotype are just tissue-culture phenomena or whether they also occur *in vivo*.

The molecular basis of transdifferentiation is now understood in several cases; for example, the conversion of pancreas to liver and the conversion of myoblasts to adipocytes. These examples generally show a close developmental relationship, perhaps making it easier to determine the genetics of the switch. But metaplasias also occur across wide developmental distances, such as in the example of bone marrow to liver. In these cases, the molecular basis is not yet known, so *in vitro* methods will probably be necessary to establish the genes that are involved and to determine whether there are intermediate steps in the process. It is not clear whether particular stem cells, such as haematopoietic stem cells, are more prone to metaplasia, or whether the experiments rely on grafting several cells, a few of which become reprogrammed when surrounded by host cells of a different type.

Finally, understanding the rules for the molecular basis of metaplasia is crucial for rational progress in the area of therapeutic stem-cell transplantation; a technology that is certain to attract considerable attention in the next few years.


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calbindin-D28K | *Cdx2* | C/EBPα | C/EBPβ | Cre | FAH | FGF-2 | β-galactosidase | GFAP | GFP | Myf-5 | MyoD | myogenin | *Pax-6* | PPAR-γ | *sine oculis* | *Six-3* | TCF4 | transferrin | transthyretin | Wnt-10b

**FURTHER READING**

**Goodell lab (protocols for the isolation of bone marrow stem-cell purification and stem cells from murine muscle):** http://www.bcm.tmc.edu/genetherapy/goodell/new_site/index2.html

**Grompe lab homepage:** http://www.ohsu.edu/somgenetics/grompe/

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