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Review Article

Adult stem cell plasticity

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Abstract

Observations made in the last few years support the existence of pathways, in adult humans and rodents, that allow adult stem cells to be surprisingly flexible in their differentiation repertoires. Termed plasticity, this property allows adult stem cells, assumed, until now, to be committed to generating a fixed range of progeny, to switch, when they have been relocated, to make other specialized sets of cells appropriate to their new niche. Reprogramming of some adult stem cells can occur *in vivo*; the stem cells normally resident in bone marrow appear particularly flexible and are able to contribute usefully to multiple recipient organs. This process produces cells with specialized structural and metabolic adaptations commensurate with their new locations. In a few examples, the degree of support is sufficient to assist or even rescue recipient mice from genetic defects. Some studies provide evidence for the expansion of the reprogrammed cells locally, but in most it remains possible that cells arrive and redifferentiate, but are no longer stem cells. Nevertheless, the fact that appropriately differentiated cells are delivered deep within organs simply by injection of bone marrow cells should make us think differently about the way that organs regenerate and repair. Migratory pathways for stem cells in adult organisms may exist that could be exploited to effect repairs using an individual's own stem cells, perhaps after gene therapy. Logical extensions of this concept are that a transplanted organ would become affected by the genetic susceptibilities of the recipient, alleles that re-express themselves via marrow-derived stem cells, and that plasticity after bone marrow transplantation would also transfer different phenotypes, affecting important parameters such as susceptibility to long-term complications of diabetes, or the ability to metabolize drugs in the liver. This article reviews some of the evidence for stem cell plasticity in rodents and man. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: **stem cells; bone marrow; regeneration and repair**

Introduction

In adult organisms, each tissue and organ are generally accepted to contain a small sub-population of cells capable of self-maintenance, of indefinite proliferative potential, and with the ability to give rise to a large family of descendants with defined spectra of specialization (multipotential stem cells) [1]. This review is focused on the recently recognized plasticity of adult stem cells, their ability, when relocated, to give rise to sets of cells that are not available from them when in their original location.

Stem cells from the inner cell mass (ICM) of the blastocyst are thought to have the greatest range of potential; these cells are essentially *pluripotential*, capable of giving rise to cells found in all three germ layers of the embryo, and this property along with certain requisite technological steps has allowed embryonic stem cells to be used to engineer strains of mouse, pig, sheep, and potentially humans. There are serious ethical concerns over the use of human embryonic stem (ES) cells, although several dozen lines have been made. Some of these ethical considerations would not apply if multipotent cells could be derived from a person for their own use. Human somatic cell nuclear transfer studies have been reported in which very early embryos were generated after reprogramming adult cell nuclei and encouraging them to function in a denucleated ova [2]. Such techniques might be used to generate cultures of multipotential cells to use for repair of that person's own tissues [3].

For many years, therapeutically useful adult stem cells have been harvested from bone marrow (either whole bone marrow in aspirates, or following mobilization and elutriation), and more recently from umbilical cord blood. Such cells are used to great effect in various haematological malignancies to replace an individual's haematopoietic stem cells (HSCs), after ablation of the pre-existing stem cell population. Some 30 years ago, it had been suggested that there were probably circulating endothelial precursor cells, derived from the bone marrow [4], yet until recently it

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had not been appreciated that bone marrow also contributed cells to tissues in other organs. The advent of robust methods for tracking cell lineage has allowed us to identify this contribution. These studies have revealed, in routine histological sections, the origin of individual cells by showing the presence of chromosomes or expression of epitopes known to be confined to the donor or recipient, e.g. Figure 1.

Of course, it is one thing for a circulating cell to engraft in another organ and assume some or all of the phenotypic traits of that organ – transdifferentiation, the acquisition of a new phenotype – but it is quite another to claim that the engrafted cell has become a local stem cell in its new niche. Demonstration of this would, ideally, require the isolation and transplantation of single cells that self-renew and produce a family of descendants that eventually become fully functional; these robust criteria have been met in one or two cases. However, some commentators have added that this phenomenon should be shown to occur 'naturally' in organs not forced to undergo organ degeneration before accepting that stem cells jump a lineage boundary [5]. Clearly, it is difficult to track cells without intervention and most of the studies to date involve damage consequent upon ablation of bone marrow by irradiation or chemical means, or the traumas of surgery and rejection, where organs have been transplanted and then studied some time later.

A counter-argument is that a degree of organ damage is essential to allow transdifferentiation or stem cell plasticity to take place at recognizable levels. It may be that migration of bone marrow stem cells throughout the body acts essentially as a back-up system, able *in extremis* to augment an organ's intrinsic regenerative capacity. The local stem cell compartment of the kidney is elusive; it may even be that the bone marrow is a renal stem cell compartment, the 'normal' physical separation of haematopoiesis from renal function in adult mammals preventing the generation of new nephrons, whereas in some fish haematopoiesis occurs in the kidney and new nephrons can be formed [6]. In any case, the lack of engraftment in the absence of organ damage in no way invalidates the claim that it does occur and it is largely in the clinical context of severe organ damage that one would envisage exploiting stem cells with transdifferentiating potential.

There is evidence that some adult stem cells may even be pluripotential, albeit in the context of creating chimeric animals, for example in the ability to contribute to all three germ layers in the pre-immune fetal sheep [7,8] and mouse or chick embryo [9].

In the following sections, we will present some of the evidence for adult stem cell plasticity (see also Table 1) together with comments on the types of stem cell present in the target or donor organs, although other articles in this issue of *The Journal of Pathology* contain much more detailed reviews on specific organs. We will also comment on potentially therapeutically useful stem cells, explain some of their properties during normal differentiation and their ability to contribute to major organs by transdifferentiation or plasticity, and indicate what has been done towards exploiting this.

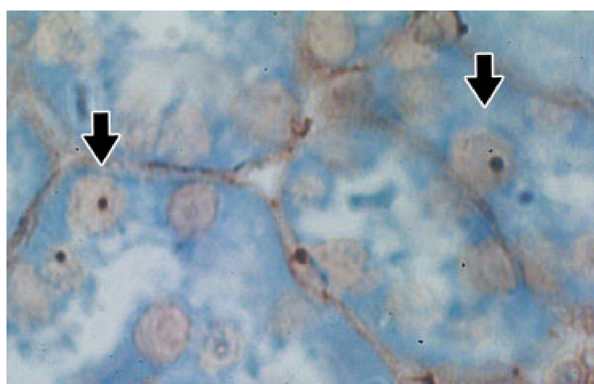

Figure 1. Section of kidney from a female mouse after bone marrow ablation and grafting with male bone marrow. Y-chromosomes were detected by hybridization *in situ* to a specific DNA paint and revealed immunohistochemically as brown dots. Tubular epithelium was demonstrated by immunohistochemistry, using an antiserum to cytochrome P450 1A2 generating a blue reaction product. Some cells within the interstitium as some tubular epithelial cells (e.g. arrows) are derived from bone marrow precursors (reproduced from the cover of *The Journal of Pathology*, vol 195, No. 2; 2001) [47]

Bone marrow

Adult bone marrow contains haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), which may derive from a common primitive blast-like cell precursor able to differentiate along MSC or HSC potentials [10].

Haematopoietic stem cells (HSCs)

The hierarchy of human haematolymphopoietic cells (HPCs) is defined by functional assays. HSCs with

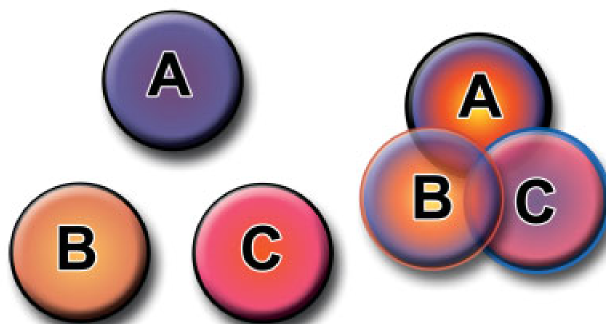

Figure 2. Representation of the established concept that there are discrete tissue-specific isolated populations of stem cells in adult organs A, B, C, *versus* the evolving concept that there is plasticity of adult stem cells, with migration between organs followed by contribution of cell numbers to maintenance and healing processes

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extensive self-renewal capacity can be assayed *in vivo* for their capacity to xenograft immunodeficient NOD/SCID mice and pre-immune sheep fetuses, models that are surrogates for syngeneic transplantation assays. Primitive HPCs with limited self-renewal potential are identified *in vitro* as high-proliferative potential colony-forming cells (HPP-CFCs). Lineage-committed HPCs with no self-renewal activity are also defined *in vitro* by clonogenic assays as colony-forming units (CFUs) or burst-forming units (BFUs).

Niches exist within the bone marrow that support HSCs, providing all requisite factors and adhesive properties to maintain their viability, and allowing an appropriate balanced output of mature progeny for the lifetime of the organism [11–14]. Niches are formed in part by the progeny of mesenchymal or marrow stromal cells (MSCs or colony forming unit-fibroblast), which supports and signals through soluble and adhesive modalities [15,16] (see reviews by Quesenberry and Becker [17] and Chan and Watt [14]).

During development, there is migration between sites capable of supporting HSCs, although in adults a homing mechanism is considered to operate that causes the majority of HSCs to return to the bone marrow within a day. A number of factors are involved in migration and homing: the ligand for c-kit, stem cell factor (SCF), particularly the cell membrane-bound variant, is important as it stimulates the adherence of HSCs to stroma. Integrin interactions are also crucial, with β-1 integrin fundamental to the migration of HSCs to the fetal liver in sheep [18], and the chemokine SDF-1 (cloned from bone marrow stromal cells) or its receptor CXCR4, essential for haematopoiesis transferring from embryonal liver to marrow in mice. SDF-1 seems to be a chemoattractant for HSCs expressing CXCR4 [19].

Clinically, advantage is taken of the ability of HSCs to migrate between stem cell compartments; G-CSF is particularly important as an agent capable for mobilizing HSCs that are then harvested for transplantation [11]. If stem cell trafficking is to be exploited to target cells to damaged organs, it is important to determine whether tissue-specific homing signals, such as selectins [20], are invoked by damage to encourage recruitment to the affected organ(s).

In some studies of adult stem cell plasticity, whole bone marrow aspirates have been injected into recipients and so the compartment responsible (HSC or MSC) for all of the novel progeny cannot be determined, but in others, exquisite effort has been invested to identify the sub-population of cells capable of integrating into specific tissues.

Mesenchymal stem cells (MSCs)

Friedenstein *et al*. reported in 1976 [21] that marrow aspirates grown at low dilution formed fibroblastic colonies: later, it was shown that they could differentiate into bone and cartilage and were transplantable [22]. Subsequently, Owen and Friedenstein [23] proposed that stromal cells from marrow in particular had the potential to generate adipocyte and osteocyte progenitors.

Approximately 30% of human marrow aspirate cells adhering to plastic are considered to be MSCs [24]. These can be expanded *in vitro* [25,26] and induced to differentiate. MSC populations are in general more difficult to characterize than the HSC populations that may be prepared using cocktails of specific antisera and FACS or magnetic beads, but it is of course necessary to demonstrate that MSC cultures are devoid of haematopoietic progenitors. The fact that adult MSCs can be expanded *in vitro* and stimulated to form either bone, cartilage, tendon, muscle or fat cells makes them attractive for tissue engineering and gene therapy strategies [27].

It is difficult to assess which factors operating during culture *in vitro* influence the plasticity of MSC cells once grafted *in vivo*: there may be bias induced during isolation of MSCs, or it may already exist *in vivo* due to the existence of regions of marrow with a propensity to differentiate along specific pathways [28,29]. It is recognized that there is significant variation between mouse strains in the yield of MSCs and their ability to differentiate along selected pathways [30] and it seems likely that variation will exist between human individuals too.

MSCs injected into the circulation can integrate into a number of tissues (see below) including, importantly, bone marrow from which they or their descendants might be released as part of a normal pattern of trafficking. Skeletal and cardiac muscle phenotypes have been reported to reside in the MSC repertoire, encouraged by pre-exposure to 5-azacytidine [31], although incompletely purified HSC populations have also been reported able to contribute to the repair of mouse tibialis anterior muscles [32], and highly purified Lin^- c-kit^+ cells are extremely efficient at generating cardiomyocytes [33].

MSC-derived cells are seen to integrate deep into brain after peripheral injection as well as after direct injection of human MSCs into rat brain; they migrate along pathways used during migration of neural stem cells developmentally, become distributed widely, and start to lose markers of MSC specialization [26]. What they become is less clear, although in related studies with mouse MSCs, some adopt neural or astrocyte phenotypes: a subset of MSC (CD11b-depleted) cells (or their progeny *in vivo*) that had been pre-labelled with BrdU *in vitro* were found to express GFAP and neurofilament markers [34]. Mouse recipients of MSCs prepared from EGFP-transgenic mice [35] were found to have a large number of EGFP-fluorescent cells in their brains; FACS analysis showed that $\sim$20% did not express CD45 or CD11b and were considered to represent a population of non-haematopoietic marrow-derived brain-specific cells. Laser scanning confocal microscopy revealed individual EGFP-positive cells with a variety of morphologies that co-expressed

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neuron-specific markers NeuN or NF-H, or the astrocyte marker GFAP [35].

Side-population (SP) cells

A numerically minor population of cells can be isolated from marrow (and other organs of several species) using fluorescence activated cell sorting (FACS) on the basis of exclusion of the fluorescent dye Hoechst 33342 [36,37]. These SP cells appear to be able to exclude xenobiotic molecules by virtue of overexpression of a number of drug efflux membrane transporter proteins and this property may confer a survival advantage [38]. SP cells have considerable potential to differentiate and integrate into other organs (mentioned below); in some circumstances, they appear uncommitted to haematopoietic lineages as they lack CD34 [39] and it is possible that they are similar to primitive blast-like cells that appear to be able to differentiate along both MSC and HSC pathways [10].

Somatic sites of engraftment

The vasculature

The endothelium of vessels in a variety of settings turns over through circulating cells and this is detectable after transplantation of organs or marked cells.

A proportion of the adult endothelium derives from circulating angioblasts; these can be harvested during the preparation of haematopoietic grafts from HSCs mobilized into peripheral blood [40] and endothelial cell progenitors isolated from circulating mononuclear blood $CD34^+$ and flk-1^+ populations [41] can differentiate into endothelial cells *in vitro*, and *in vivo*, they or their progeny contribute to neo-angiogenesis driven by ischaemic injury in mouse and rabbit models [42]. Progeny integrate into new microvessels in skin, heart, skeletal muscle, endometrium, and corpus luteum [43]. Circulating precursor numbers increase following ischaemia, or after GM-CSF pretreatments [44].

These observations help to support hypotheses formed over 30 years ago (by counting Barr bodies): that endothelial replacement in grafted organs is encouraged where endothelial damage is severe [4] and that extensive acute damage requires repair by host cells while less severely damaged grafts could be restored by endothelial continuity from surviving donor endothelial cells [45]. More recently, Lagaaij *et al*. suggested that the extent of replacement of endothelial cells lining small renal vessels was related to the severity of vascular rejection, as six of seven grafts affected by vascular rejection showed over 33% recipient-derived endothelial cells, whereas just two of 13 patients without evidence of rejection showed as extensive endothelial recolonization [46]. We have seen occasional male endothelial cells in female renal allografts [47]. However, Andersen *et al*. studied 45 renal biopsies from 40 sex-mismatched transplant patients suspected of developing *acute* rejection but found no evidence of revascularization by the recipient, even in four cases where the transplant failed [48].

The origin of glomerular endothelium in transplanted kidneys is less clear. It might be expected that migration and integration of recipient EPCs should occur in the glomerulus, yet Sinclair [45] considered glomeruli to be unaffected, Lagaaij *et al*. did not comment on them [46], and Andersen *et al*. found no recipient endothelium [48]. Only in the mouse is there firm evidence that whole bone marrow contributes to glomerular endothelium [49].

Considering large vessels, Williams *et al*. [4,50] studied endothelial repopulation of grafted aorta segments and found up to 10% of the endothelium to be host marrow-derived, and engraftment was less when rejection was attenuated by immunosuppression. Circulating cells derived from the recipients of heart allografts in mice were thought to contribute substantially to the formation of neointimal hyperplasia when acute rejection was suppressed with FK506 [51]. Whether circulating smooth muscle progenitors are recruited in large numbers or proliferation occurs from just a few was not established; others have suggested that marrow-derived cells do not contribute significantly to the newly expanded population of smooth muscle actin-expressing cells [52].

Injection of bone marrow cells (principally MSC-derived) into damaged rat heart muscle promoted angiogenesis and some of the new capillaries were MSC-derived [31]. Highly purified rat Lin^- c-kit^+ cells into infarcted rat hearts produced substantial repair via the generation of not only new marrow-derived endothelial cells, but also cardiomyocytes and smooth muscle cells [33].

Adult human $CD34^+$ bone marrow cells mobilized by G-CSF contribute to the repair of rat hearts following infarction [53]. This remarkable ability was shown to be principally due to angioblast precursors ($CD34^+$/$CD117^{Bright}$/GATA-2^{Hi}) generating new human capillaries specifically within the infarct zone that improved the salvage of rat myocytes (not generating new myocytes).

Further support for marrow-derived cells contributing to 'maintenance angiogenesis' was provided by Gunsilius *et al*. [54], studying patients with chronic myeloid leukaemia: individual endothelial cells in heart vessels of one patient were seen to bear the t(9;22) chromosomal translocation and some endothelial cells were derived from a therapeutic HSC graft, revealing that the endothelium was turned over at different stages.

In patients who have received liver transplants, repopulation of both portal and hepatic veins by endothelium of recipient origin has been 0observed. In the same report, this was also noted in a proportion of mice following bone marrow transplantation, suggesting marrow origin [55].

Bone marrow cells also contribute to tumour

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angiogenesis in a transplantable murine neuroblastoma [56]. This was exploited therapeutically: using marrow cells transduced with a truncated soluble vascular endothelial growth factor receptor-2 (tsFlk-2) reduced tumour vascularity and slowed tumour growth [56].

Heart

Cardiomyocytes appear to have a modest capacity for self-renewal in areas adjacent to infarcted myocardium [57]. A number of studies have shown cardiomyocyte differentiation to occur *in vitro* or *in vivo* from cell lines, circulating cells or directly from bone marrow.

Six weeks after direct injection of the cell line WB-F344, clonally derived from a young male rat liver and tagged with the *E. coli* lac Z gene, into the left ventricle of female nude mice, donor cells showed cardiomyocyte differentiation, as they expressed cardiac troponin T and formed intercalated discs with host myocytes; the donor origin of the cells was confirmed by the presence of the rat Y-chromosome and the expression of *E. coli* β-galactosidase [58].

Adult mouse MSCs in culture can generate spontaneously beating cardiomyocytes [59] and there is evidence for the generation of mature cardiac myocytes *in vivo* derived from adult stem cells. Tomita *et al.* [31] found that injection of BMCs into cryo-scarred hearts *in vivo* induced angiogenesis, but only BMCs cultured with 5-azacytidine (to induce differentiation into cardiac-like muscle cells) were able to integrate within ventricular scar tissue and improve myocardial function.

Cells derived from sorted bone marrow cells can also differentiate into cardiomyocytes. In female mice, direct injection of Lin^- $c\text{-}kit^+$ bone marrow cells (from male EGFP transgenic donors) into the contracting area bordering an experimental infarct results in more than half the infarcted area being colonized by donor cells within 9 days: male EGFP-positive cells had proliferated *in situ* and expressed proteins characteristic of cardiac tissue, including connexin 43, suggesting intercellular communication [33]. Mouse haematopoietic SP cells are also able to make heart muscle and endothelial cells [60].

Bittner *et al.* [61] used male wild-type bone marrow and spleen cells to treat female mdx mice; in this model of Duchenne muscular dystrophy, they observed occasional male cardiomyocytes within the cardiac muscle syncytium and a few male endothelial cells in cardiac vessels.

In contrast, Pereira *et al.* [62] found no evidence of MSC-derived cells in the heart or aorta of mice 2.5 months after intraperitoneal injection and Kocher *et al.* [53] reported that no marrow-derived cardiac myocytes were detectable in rats after tail vein injection of adult human $CD34^+$ bone marrow cells into rats with infarcts, although it may be that the nature of the lesion promoted incorporation and differentiation into an exclusively angioblast direction.

In humans, a series of patients suffering from myocardial infarction, who were treated by coronary angioplasty and injection of autologous bone marrow into their coronary arteries, were reported to show a functional benefit following this treatment [63]. Based on observations in rodent models, this benefit could be from microvascular improvements alone, although the technique of Y-chromosome detection in sex-mismatched cardiac transplants revealed that a small proportion of cardiomiocytes were of extracardiac origin, as well as coronary arterioles and capillaries [64]. These observations reinforce our view that many organs are subject to a flux of circulating stem cells and that this may augment organ function.

Bone marrow cells can also contribute in a more subtle way to restoration of cardiac function after myocardial infarction: labelled $CD34^+$ human bone marrow cells were injected intravenously into athymic nude rats with an experimental myocardial infarction and found to enhance infarct zone microvascularity and reduce ventricular remodelling, a process which, if left unchecked, precipitates heart failure [53].

Lung

The bronchopulmonary tree is lined throughout by epithelial cells and indigenous multipotential stem cell populations have been proposed to exist at several levels, based on observations made after cell injury. In the pseudo-stratified epithelium lining the proximal airways (trachea and bronchi), the so-called basal cells appear to be the major proliferative cells. In the terminal and respiratory bronchioles, the dome-shaped Clara cells show an enhanced proliferative rate after injury [65], whereas in the alveoli the cuboidal-shaped type II pneumocyte appears to be the stem cell able to proliferate and generate differentiated progeny, notably the type I (squamous) pneumocyte.

In terms of plasticity, it has been claimed that even a single cell from a male bone marrow population (lineage-depleted and enriched for $CD34^+$ and $Sca\text{-}1^+$ by *in vivo* homing to the bone marrow) can, when injected into female recipients along with 2×10^4 female supportive haematopoietic progenitor cells, give rise to a variable proportion of epithelial cells in some organs: at 11 months, a surprisingly high proportion (20%) of cytokeratin-expressing alveolar pneumocytes were Y-chromosome-positive (many identified as type II cells by surfactant B synthesis), with somewhat fewer ($<4\%$) Y-chromosome positive bronchial epithelial cells [66]. The high level of lung engraftment was conjecturally attributed to either lung damage caused by the 'lethal' irradiation to permit bone marrow transplantation, or viral infection in the temporarily immunosuppressed animals.

Somewhat different observations were made by Kotton *et al.*, who injected plastic-adherent cultured bone marrow cells intravenously into recipient mice 5 days after alveolar injury induced by bleomycin [67]. The lung was the only organ with any engraftment and, as one might expect, injury promoted this process,

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but surprisingly between 1 and 30 days after injection they found only type I rather than type II pneumocytes (considered stem cells for type I) of donor origin. In this study, donor-derived cells were found in occasional clusters, but an absence of proliferative activity amongst these cells may suggest many cells engrafted into a particular site, rather than clonal proliferation. The short-term seeding of lung by intravenous bone marrow can be reduced by a vasodilator [68].

Gastrointestinal tract

The mucosa of the gastrointestinal tract has well-characterized stem cell regions: in gastric body glands, stem cells are just below the foveolas. It is widely accepted that these stem cells are multipotential, capable of giving rise to all the indigenous lineages [69].

Chimeras generated in chick or mouse embryos revealed that adult mouse brain neural stem cells are able to be reprogrammed and to contribute to the liver and intestine [9].

Fetal mouse liver (ED13.5) also contains a population of highly clonogenic cells (for liver), but when injected into the duodenal wall the cells can apparently form villus and cryptal epithelial cells [70].

In adult mice, there is some evidence supporting the integration of marrow-derived cells into 'functional' epithelial cells in the oesophagus, stomach, and small and large bowel. Eleven months after engraftment of a single male haematopoietic stem cell, Krause *et al.* [66] found that between 0.19% and 1.81% of cells within the GI tract were HSC-derived; these cells strongly resembled indigenous gut lineage cells: e.g. morphologically appropriate absorptive villus epithelial cells were cytokeratin-positive. Despite being appropriate to the nearby 'stem cell niches' [71] in colonic crypts and gastric glands, there was no evidence that these cells were part of a local tissue-specific clonal stem cell population. It remains possible that these cells had differentiated directly from a circulating multipotential cell, derived from the single cell injected.

Transplantation of male whole bone marrow into female recipients is followed in the small and large intestine by progressive conversion (to male) of the smooth muscle actin-expressing myofibroblast population surrounding crypts [72] (and elsewhere in this issue), again supportive of a flux of cells through many organs.

Liver

The liver can call upon indigenous populations of both functional stem cells and potential stem cells [73] with which to respond to injurious agents or damage. The response to parenchymal cell loss is that the hepatocytes normally restore the liver mass, rapidly re-entering the cell cycle from the G_0 phase. Even after a two-thirds partial hepatectomy, the remaining cells need to cycle only two or three times to restore pre-operative cell number. Hepatocyte transplantation protocols, developed because of the shortage of livers for whole-organ transplantation, have shown that transplanted cells can undergo significant clonal expansion within the recipient's diseased liver; thus, at least some hepatocytes can be considered true functional stem cells. If either massive damage is inflicted upon the liver or regeneration after damage is compromised, a *potential* stem cell compartment residing within the smallest branches of the intrahepatic biliary tree is activated; this is seen as the 'oval cell response' or 'ductular reaction'. This amplifies the biliary population before these cells differentiate into hepatocytes [74,75].

One of the first demonstrations of stem cell plasticity was in the liver. It was recognized that oval cells can express antigens traditionally associated with haematopoietic cells (including c-kit, flt-3, Thy-1, and CD34). This led to the suggestion that perhaps bone marrow cells were at one end of a common differentiation spectrum, with hepatocytes at the other end. Oval cells/hepatocytes were first discovered to be derived from circulating bone marrow cells in the rat by Petersen *et al.* [76], who followed the fate of syngeneic male bone marrow cells transplanted into lethally irradiated female recipient animals whose livers were subsequently injured by a regime of 2-acetylaminofluorene (to block hepatocyte regeneration) and carbon tetrachloride (which causes hepatocyte necrosis) designed to cause oval cell activation. Y-chromosome-positive oval cells were found 9 days after liver injury and some Y-chromosome-positive hepatocytes were seen after 13 days when oval cells were differentiating into hepatocytes. Additional evidence for hepatic engraftment of bone marrow cells came from a rat whole-liver transplant model in which Lewis rats expressing the MHC class II antigen L21-6 were made recipients of livers from Brown Norway rats that were negative for L21-6. Subsequently, ductular structures in the transplants contained both L21-6-negative and L21-6-positive cells, indicating that some biliary epithelium was of *in situ* derivation and some was of recipient origin, presumably from circulating bone marrow cells [76].

Using a similar gender-mismatch bone marrow transplantation approach in mice to track the fate of bone marrow cells, Theise *et al.* reported that over a 6-month period 1–2% of hepatocytes in the murine liver may be derived from bone marrow in the absence of any obvious liver damage, suggesting that bone marrow contributes to normal 'wear and tear' renewal [77]. It was thought unlikely that the bone marrow transplant contained a liver progenitor cell that was not of bone marrow origin, since 200 $CD34^{+}$ lin^{-} marrow cells produced the same degree of hepatic engraftment as 20 000 unfractionated bone marrow cells.

Importantly, the ability of bone marrow cells to cure a metabolic liver disease has been shown in FAH minus mice (causing type 1 tyrosinaemia – see above)

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Table 1. Examples of adult stem cell plasticity, based on lineage tracking and phenotype determination

Recipient organ	Donor cells	Cell type	Proof of donor origin/proof of new phenotype	Reference
Liver (rat)	Bone marrow	Oval cells, hepatocytes	ISH & MHC class II antigen L21-6/morphology	76
Liver (human)	Bone marrow	Hepatocytes	ISH/CK8 or albumin	80,81
Liver (human)	Extra-hepatic	Hepatocytes	Laser capture of CK$^+$ cells, genotyping	82
Liver (mouse)	KTLS cells	Hepatocytes	β-gal/FAH$^+$/morphology	78
Liver (mouse)	Pancreatic exocrine cells	Hepatocyte	GFP/G-6-P & transferrin	90
Liver (mouse)	Pancreas	Hepatocyte	ISH/FAH$^+$	91
Liver (mouse, human)	Bone marrow	Endothelium	ISH/Factor VIII	55
Kidney (mouse, human)	Bone marrow	Tubular epithelium, glomeruli	ISH/cytochrome P450 & CAM 5.2	47
Kidney (mouse)	Bone marrow	Mesangial cells, endothelium	Morphometry, culture, genotype, MMP assay	49
Kidney (human)	Bone marrow	Endothelium	XX chromosome & HLA typing/morphology	46
Kidney (human)	Extrarenal	Endothelium	Barr-body detection/morphology	4
Heart (mouse)	Bone marrow	Myocardium	ISH & GFP/cardiac myosin	33
Heart (mouse)	Bone marrow SP cells	Cardiomyocytes and endothelium	β-gal/cardiomyocytes: α-actinin and endothelial cells: flt-1	60
Lung (mouse)	Bone marrow	Type II pneumocytes	ISH/surfactant B	66
Lung (mouse)	Bone marrow-plastic adherent	Type I pneumocytes	β-gal/morphology	67
Marrow (mouse)	Neuronal	Multiple haematopoietic lineages	β-gal/morphology	106
Bone (mouse)	Bone marrow (also MSCs)	Collagen-expressing cells	Expression of minigene	62
Bone (human)	Bone marrow	Osteoblasts	ISH/morphology and culture	126
CNS	Bone marrow	Neurones	ISH/NeuN	110
CNS	Bone marrow	Microglia and astrocytes	ISH & GFP/macrophage antigen F4/80	111

ISH = *in situ* hybridization for Y-chromosome; CK = cytokeratin; G-6-P = glucose-6-phosphatase; β-gal = β-galactosidase; GFP = green fluorescent protein; FAH = fumaryl acetoacetate hydrolase; GFAP = glial fibrillary acidic protein.

[78], thus establishing haematopoietic cells as a fully functional stem cell population for hepatocytes. While it seems logical to believe that parenchymal damage is a stimulus to hepatic engraftment by HSCs, the molecules that mediate this homing reaction to the liver are unknown. Petrenko *et al*. speculated that in mice the molecule AA4 (the murine homologue of the C1q receptor protein) may be involved in the homing of haematopoietic progenitors to the fetal liver – maybe this receptor protein is expressed on haematopoietic stem cells that engraft to the damaged liver [79]. Clearly there may well be multiple other signals mediating this engraftment.

Two groups have demonstrated that some hepatocytes are derived from bone marrow cell populations in humans [80,81]. Two approaches were adopted: in one, the livers of female patients who had previously received a bone marrow transplant from a male donor were examined for cells of donor origin using a DNA probe specific for the Y-chromosome, localized using *in situ* hybridization; in the second, Y-chromosome-positive cells were sought in female livers engrafted into male patients but which were later removed or biopsied for recurrent disease. In both sets of patients, Y-chromosome-positive hepatocytes were readily identified. Hepatic engraftment of HSCs into human liver was highly variable, perhaps related to the severity of parenchymal damage, with up to 40% of hepatocytes and cholangiocytes derived from bone marrow in a liver transplant recipient with recurrent hepatitis C. Recently, a study of 27 sequential biopsies from nine liver transplant recipients found that whereas biliary epithelial chimerism was a consistent feature of most biopsies, hepatocyte chimerism was more prominent in those patients suffering recurrent hepatitis, again suggesting that local organ damage is necessary for significant engraftment of circulating stem cells into the liver [82].

Pancreas

The pancreas is composed of an exocrine portion organized into acini and secretory ducts and an endocrine portion organized into islets of Langerhans, which contain the β-cells involved in homeostasis of plasma glucose levels. Until recently, it was thought that a person was born with all the pancreatic β-cells that they would ever have, but it is now apparent that there are low levels of mature β-cell replication and apoptosis in adults, so the β-cell population should be defined as a slowly renewing population [83]. Multipotential stem cells appear to be located in the pancreatic ducts, with the potential to generate endocrine, acinar, and ductular cell phenotypes [84], and

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even giving rise to new islets (islet neogenesis) when presented with a functional demand. Mature duct cells are likely to be *potential* stem cells, able temporarily to exhibit a less differentiated phenotype, expand, and subsequently differentiate along any one of the pancreatic lineages [85]. In keeping with this concept, functional islet β-cells have been generated *in vitro* from cultured pancreatic ductal cells [86]. Recently, Zulewski *et al*. suggested that there exists in both islets and ducts, a sub-population of cells expressing the neuronal stem cell marker nestin that are the true stem cells; *ex vivo*, these cells are highly clonogenic and can differentiate not only into endocrine and exocrine pancreatic cells, but also into cells with a hepatic phenotype [87].

During development, the ventral pancreas and liver emerge from the same general area of ventral foregut endoderm, but FGF from the cardiac mesoderm inhibits pancreatic development in the presumptive liver [88]. Thus, it is not surprising that certain pancreatic cells can transdifferentiate into hepatocytes; another example of pancreatic-liver cellular plasticity was afforded by Krakowski *et al*., who generated insulin promoter-regulated keratinocyte growth factor (KGF) transgenic mice. Under the influence of KGF, numerous functional hepatocytes emerged within the islets of Langerhans [89]. A combination of dexamethasone and oncostatin M (a natural hepatocyte differentiation factor produced by haematopoietic cells in the fetal liver) is a very effective *in vitro* inducer of pancreatic exocrine cell transdifferentiation into hepatocytes [90]. This differentiation was associated with the induction of the transcription factor C/EBPβ, a factor thought to accelerate fatty acid acyl CoA synthesis. This in turn bound to HNF4, causing its translocation to the nucleus, where it activated genes such as α-fetoprotein and transthyretin that are normally switched on during early hepatocytic differentiation.

In the FAH-deficient mouse model of type 1 tyrosinaemia, transplantation with pancreatic cells is generally not life-saving, but a small proportion of animals do survive, with 50–90% replacement of the diseased liver by pancreatic cell-derived hepatocytes [91]. Given that animals fed a copper-deficient diet undergo pancreatic exocrine cell atrophy and that refeeding induces the surviving ducts to give rise to hepatocytes, it was surprising that pancreatic cell suspensions enriched for pancreatic ducts were poorer than unfractionated pancreatic cells at reconstituting the diseased FAH–/– liver with functional hepatocytes [92]. Moreover, we have already noted that the pancreatic ducts appear to be the location of multipotential stem cells, at least for pancreatic lineages; however, human pancreatic exocrine cells *in vitro* readily assume a ductal phenotype and re-express PDX-1 (pancreas/duodenal homeobox 1), a homeodomain protein essential for pancreatic ontogeny [93]. Thus, exocrine cells can be a source of multipotential stem cells for the pancreas and this flexibility seems to extend to hepatic lineages both *in vitro* and *in vivo*.

Kidney

The kidney has no recognizable stem cell zone, but tubular cells can regenerate after injury. Adult mouse neural stem cells injected into an early embryo contribute to the developing kidney [9], so adult cells may be reprogrammed to differentiate into renal cells.

In the kidney, a conversion or 'transdifferentiation' has been suggested to occur between the phenotype of epithelial cells and fibroblasts (both being generated originally from the primitive metanephric mesenchyme) that is a response to a breach of the tubular basement membrane [94]. In various models, epithelial cells are seen to acquire markers of fibroblasts or myofibroblasts and adopt a fusiform morphology; in interstitial fibrosis, cells are seen with a fibroblastic morphology, yet they bear epithelial markers. Transdifferentiation appears restricted to regions where the basement membrane is damaged, with the most myofibroblastic cells seen where the tubular basement membrane was extensively damaged [95]. These observations, and others on cultured cells, are concordant with the hypothesis that the epithelium adopts a fibroblastic morphology before proliferating and perhaps before helping to repair the basement membrane. Sun *et al*. [96] examined rat kidneys after uranyl acetate-induced tubular necrosis and considered that repair occurred without movement of cells from the interstitium into the denuded tubules, yet they observed proliferation of flattened cells lining the regenerating tubules that expressed vimentin, like myofibroblasts.

An alternative interpretation is that the myofibroblasts are of extrarenal, perhaps normally bone marrow, origin. There is some support for this view. In our studies of whole bone marrow transplants in mice, we observed marrow-derived renal tubular epithelial cells [47] and in human renal transplants, where female kidneys were grafted into male recipients, we noted male tubular cells expressing the epithelial marker CAM 5.2 [47]. Furthermore, Grimm *et al*. found evidence for a circulating host-derived mesenchymal cell in renal transplants that were suffering chronic rejection [97]. Not all studies have come to the same conclusions; Andersen *et al*. [48] reported that tubular and glomerular cells remained of donor origin in transplanted kidneys even 10 months after transplantation, and no donor-derived renal tubular epithelial cells were seen in any of the five mice grafted by Krause *et al*., perhaps due to the use of sorted haematopoietic stem cells, rather than whole marrow cells [66].

Considering glomeruli, we have described marrow-derived cells that appeared to be podocytes [47], although Krause *et al*. did not see any [66] and Grimm *et al*. concentrated on the interstitium [97].

Several studies have demonstrated that bone

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marrow-derived cells can affect the progression of renal disease. Yokoo *et al.* used engineered bone marrow to deliver to the glomerulus, cells expressing a gene that reduced susceptibility to experimental Goodpasture's syndrome [98], although they did not seek to establish whether any 'plasticity' had occurred. One of the most significant reports of bone marrow-derived cells contributing to renal repair is that of Cornacchia *et al.*, who demonstrated that bone marrow from mice with an inherited glomerular mesangial sclerosing defect transferred the disease phenotype; the morphology and MMP expression levels were due to the generation of endothelial and mesangial cells from the donor bone marrow [49].

Nervous system

The mammalian brain develops as a tube containing a ventricular compartment filled with cerebral fluid. During development, the dividing cells are located in the cell layer that lines the lumen of the neural tube (corresponding to the localization of the ependymal cells in the adult). These cells show tri-lineage potential capable of differentiating into either astrocytes, oligodendrocytes, or neurons.

In the adult, single cells isolated from the lining of the ventricular system (ependymal cells and cells from the subventricular zone [99], where it exists) are capable of forming spheroids of tightly clustered cells (neurospheres) that show the same tri-lineage potential [100–102]. The existence of multipotential neural stem cells *in vivo* is now widely accepted and growth factors such as EGF and FGF-2 are significant players in their self-renewal [103,104]. Furthermore, cells with considerable replication potential and the ability to form astrocytes and neurons can be isolated from human post-mortem tissue [105].

More intriguingly, Bjornson *et al.* demonstrated that single neural stem cells with tri-lineage potential could transdifferentiate into several haematopoietic lineages in mice [106]. An *in vitro* clonogenic assay of the bone marrow from the transplanted mice showed that some of the colonies were positive for β-galactosidase, suggesting neural stem cell origin. Significantly, cultured neural stem cells neither proliferated nor formed haematopoietic progeny in the same clonogenic assays without prior injection into the irradiated hosts, indicating that an appropriate microenvironment is necessary for transdifferentiation. Likewise, clonally-derived human neurosphere cells derived from fetal tissue and expanded *in vitro* by EGF and/or FGF-2 show no haematopoietic potential in culture, but can establish long-term haematopoiesis in human bone fragments in SCID-hu mice [107]. However, a recent study using a similar protocol to Bjornson *et al.* rigorously tested the haematopoietic potential of murine neurosphere cells and was unable to find any evidence of haematopoietic differentiation in a large group of sub-lethally irradiated mice, which suggests that haematopoietic potential is not a general property of neural stem cells [108].

In mouse : chick chimeras, created by the injection of neurospheres from ROSA26 mice, β-galactosidase-positive cells contributed to several tissues including liver, gastric mucosa, and mesonephric tubules [9]. Thus, neural stem cells have a considerable flexibility, but it has not been proven that these pathways of differentiation exist normally. In particular, they would not satisfy one of the criteria stipulated by Anderson *et al.* [5], namely that the donor population should be transplanted without intervening culture manipulations, since all studies used cells from cultured neurospheres.

Neural stem cells have other potentialities; clonally-derived murine and human adult neural stem cells can undergo apparent myogenic differentiation *in vitro* when co-cultured with myoblasts, suggesting that mature tissues can provide epigenetic signals to neighbouring cells to undergo alternate pathways of differentiation [109]. Moreover, a small percentage (1–2%) of such cultured cells could undergo myogenic differentiation when injected into cardiotoxin-damaged skeletal muscle. Likewise, neural stem cells from ROSA26 mice, co-cultured with ES cell-derived embryoid bodies are able to show myogenic differentiation [9]. Impressive as all these data might seem with multiple lines of evidence proffered for myogenic conversion, these in no way provide evidence that neural stem cells can act as stem cells for skeletal muscle and there is no real expectation that a trafficking from the brain to skeletal muscle occurs *in vivo*.

Looking for plasticity in the other direction, it is readily apparent that cells from *outside* the nervous system can differentiate into neurons and glial cells. Mezey *et al.*, studying the homozygous PU.1 mutant female mouse (PU.1 is a transcription factor required for the histogenesis of six of the haematopoietic lineages), 'rescued' these mice with a life-saving bone marrow transplant from male wild-type donors and then found that up to 4.6% of cells in the CNS were Y-chromosome-positive and that up to 2.3% of Y-positive cells possessed the neuronal markers NeuN and neuron-specific enolase (NSE) [110]. Similarly, Eglitis and Mezey detected significant numbers of microglia (F4/80-positive) and astrocytes (GFAP-positive) of bone marrow origin in the brains of recipient female mice 6 weeks after transplantation of male bone marrow [111]. Intraperitoneal injection of whole bone marrow from mice transgenic for a green fluorescent protein was seen to engraft many organs, and effect a partial rescue of the Twitcher phenotype, by generating many fluorescent/Mac1-positive cells within the CNS and spinal cord and extending the lifetime of these mice from ~45 to over 100 days [112].

Marrow stromal cells (MSCs) may also be able to differentiate along CNS lines, as ventricular transplantation of myelo-depleted MSCs (marked by bromodeoxyuridine in culture) resulted in their widespread

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distribution by 12 days post-transplant, and some labelled cells were either neurofilament- or GFAP-immunopositive [34]. MSCs *in vitro* can also be induced to differentiate along neuronal lines, with the cells having neuronal morphology and being initially nestin-positive (characteristic of neuronal precursors) before expressing typical neuronal markers such as NSE and NeuN [113]. Given the inaccessibility of conventional neuronal stem cells, MSCs may therefore eventually have application in the treatment of neurodegenerative disease.

Skin

The epidermis and hair follicles are prime examples of tissues that require a high capability of self-renewal. In normal epidermis, proliferation is confined to the basal layer that contains both the stem cells and the more numerous transit-amplifying cells. In thin rodent epidermis, the suprabasal cells are arranged in columns (stacks) that interdigitate with neighbouring stacks, each stack being associated with a seemingly defined group of basal cells, and a more slowly dividing cell underneath the centre of each stack has been proposed as the stem cell for the so-called epidermal proliferative unit (EPU). Human epidermis is much thicker and is generally not stacked, and the identity of stem cells is more controversial, though markers such as β-1 integrin expression have been proposed [114]. In most areas of the epidermis, this is confined to the tops of the dermal papillae [115]. The basal cells of the interfollicular epidermis are continuous with those of the hair follicle and here multipotential stem cells are tucked away in the permanent portion of the follicle called the bulge. Bulge cells have the classic stem cell properties of low *in vivo* proliferation and high *in vitro* clonogenic potential. Elegant experiments involving creating chimeric vibrissal (whisker) follicles by transplanting the bulge region from ROSA26 mice to wild-type mouse follicles have shown that bulge cells migrate both downwards and upwards, forming all follicular, sebaceous, and epidermal lineages [116].

In terms of plasticity, the study of Krause *et al.* indicated that haematopoietic cells in the female mouse could differentiate into cytokeratin-positive epidermal cells; they found, using Y-chromosome tracking techniques, that approximately 2% of epidermal cells were Y-chromosome-positive 11 months after HSC transplantation. No clonal proliferation of such cells was seen, though the authors illustrate one such cell as possibly being located in the bulge region [66].

Combining murine embryonic dermis with rabbit central corneal epithelium causes the transit-amplifying cells, thought to be located here, to be reprogrammed [117]. Multipotential cells have been isolated from rodent and human skin, specifically from the dermis, and named SKPs – skin-derived precursors [118]. These cells can undergo multiple rounds of cell division and be instructed to undergo differentiation along neuroectodermal lines (neurons and glial cells) or mesodermal lines (adipocytes and smooth muscle). These cells were distinguishable in their behaviour from plastic-adherent bone marrow mesenchymal cells, and apparently clonally-derived spheres of these cells could generate all the above lineages, so perhaps they are equivalent.

Some murine epidermal cells may even be pluripotential; isolation of epidermal stem cells on the basis of size and Hoechst 33342 dye exclusion from 3-day-old eGFP transgenic mice, and their injection into wild-type blastocysts, results in their incorporation into a variety of tissues in all three germ layers [119].

Wounds created by clipping the tail of fetal sheep at the same time that adult human MSCs were injected intraperitoneally were found subsequently to have human cells of fibroblastic morphology within the dermis and dermal appendages [7]: this might indicate that circulating MSCs have the potential to assist with skin repair processes.

Skeletal muscle

Satellite cells are responsible for the maintenance of muscle fibres and are the local stem cells, able to divide and self-renew. They are mononuclear cells, located normally between the sarcolemma and the basal lamina of the muscle fibre.

When purified from adult mouse skeletal muscle, cultured, and then injected into mice (along with other distinguishable whole marrow), satellite cells generated a full-range multi-lineage engraftment of the HSC compartment [36]; a further round of bone marrow grafting successfully transferred these lineages to other mice. Several reports indicate that a common haematopoietic and muscle precursor exists in adult muscle and in marrow, and it is interesting to speculate that the SP fraction of many tissues contains a population of multipotent or pluripotent stem cells.

The fact that purified human muscle myoblasts, injected directly into the muscle of patients with Duchenne muscular dystrophy, can integrate into myotubes and express muscle-specific transcripts was shown by Gussoni *et al.* [120]. Subsequently, that team used the mdx mouse model of muscular dystrophy to establish that intravenous injection of wild-type male HSCs, or SP cells isolated from muscle resulted in the integration of male nuclei into female mdx mouse myotubes with $\sim$1% expressing dystrophin [121]. Ferrari *et al.* [32] demonstrated that whole bone marrow contains cells able to migrate into damaged skeletal muscle and within weeks contribute nuclei to myotubes. A muscle-derived clonal cell population (mc13) expressing both muscle and stem cell markers was shown to integrate at low efficiency into muscle after intravenous injection into mdx mice; integration was greater if the cells were injected directly into the dystrophic muscle [122].

Bittner *et al.* [61] demonstrated in female mdx mice that male donor marrow/spleen cells or their progeny invaded skeletal muscle and contributed to endothelial

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and myotube populations; Y-chromosome-positive nuclei were seen within dystrophin-expressing myotubes. Integration occurred whether or not the recipient bone marrow was ablated by irradiation. Human skeletal muscle cells (hybridizing to an *Alu* I DNA probe and expressing dystrophin, or expressing human β-2 microglobin and fast or slow myosin) were detected 5 months after injection of adult human MSCs into fetal sheep [7].

Neural stem cell 'neurospheres' of human or mouse origin are also able to contribute to skeletal muscle fibres *in vivo* after transplantation into adult mice [109] and can form myotubes after physical contact and co-culture with C2C12 cells that themselves showed myotube formation. The fact that myotubes contain several nuclei raises the possibility that the formation of heterokaryons might confound other studies that seem to support stem cell plasticity (discussed below).

Bone

Whole male mouse marrow, injected intravenously into female mice that have not had their marrow ablated, contributes differentiated cells to long bones, albeit at a low frequency [123]. The Y-chromosome-positive cells seen in the bones were considered to be functionally active as osteoblasts, producing bone before becoming encapsulated within the bone lacunae and terminally differentiating into osteocytes. Y-positive flattened bone lining cells on the periosteal bone surface were also present.

The important principle that MSCs can be used to direct tissue-specific gene expression was shown clearly by Hou *et al.* in mice [29]. They used a reporter gene under an osteocalcin promoter; after intravenous injection, MSCs, or their progeny, were found throughout a wide range of tissues, but *expression* of the reporter gene was found only within bony tissues, confined to a subset of osteoblasts and mature osteocytes within well-formed lacunae.

A variety of therapeutic protocols were examined using a mouse model of osteogenesis imperfecta (OI) [62,124], a genetic disorder of one of the genes for collagen I chains that form the primary protein scaffold for bone formation, frequently resulting in a generalized osteopenia, fragile bones, and short stature. These studies assessed the extent of engraftment of tissues with wild-type MSCs or whole marrow cells (WSCs) and sought to detect any improvement in bone composition. When MSCs expanded from mice transgenic for a human collagen I minigene, injected these cells intravenously into recipient wild-type mice and found that cellular progeny had infiltrated a variety of wild-type mouse tissues: the frequency of MSC-derived cells within the organs increased over time and expression of mRNA from the minigene was seen in bone but not cartilage (and could not be expressed from HSC progeny). The recipient's lungs appeared to contain large numbers of MSC progeny, but expressed the mRNA at a lower level. The authors hypothesized that bone cells derived from the transplanted marrow would have a selective advantage over the resident OI cells, as OI MSCs were defective in differentiation towards an osteoblast phenotype *in vitro*. Three-week-old female OI mice given several injections of male MSCs intraperitoneally were found 1 month later to have small but significant increases in bone collagen and mineral content; after 2.5 months, male MSC-derived cells were detected by FISH in primary cultures from one OI mouse: male cells comprised 7% of long bone and 15% of calvaria cells; however, the phenotype of these cells was not determined.

Therapeutic intervention has been attempted in infant patients with OI. In a 'proof of principle' study [125], patients were given whole bone marrow grafts after ablation of their original marrow; after 3 months, all three initial patients showed an increase in total body bone mineral content, associated with improved growth and less fracturing. Cultures of a trabecular bone biopsy from a female patient receiving male marrow revealed that $\sim$1.5% of osteoblasts were donor-derived [126]; it is not obvious how such a low level of engraftment could have the substantial benefits described. Subsequently, additional grafts of MSCs from the original donors were given to see if more osteoblasts could be replaced [127].

A potential complication of studies of bone growth and turnover in which growth or mineralization effects are attributed to MSC grafts is that osteoclasts, the primary cells responsible for resorption of bone normally and in pathological conditions, arise from precursors of the monocyte/macrophage lineage elaborated by haematopoietic stem cells (HSCs). Another complication is that a population of non-adherent low-density cells exist in marrow that have the ability to promote bone precursor development through the release of soluble factors [128]; these cells would be depleted in most MSC culture protocols. The balance between osteoblast and osteoclast formation may affect growth and might offer an avenue for some therapeutic interventions: the PPAR-γ pathway is active in differentiation of both HSCs to osteoclasts [129] and MSCs to osteoblasts [130].

Muscle stem cells have been isolated and clonal populations produced that yield bone *in vitro* on exposure to bone morphogenetic protein 2 (BMP-2). Further, adenoviral-transduced expression of BMP-2 by these cells allows them to make ectopic bone after intramuscular injection, or heal skull bone damage [122].

Cartilage

Key factors involved in the differentiation of MSCs towards mature cartilage are becoming identified through *in vitro* studies of isolated and expanded MSCs: they can be induced by dexamethasone and TGFβ3 to secrete an extracellular matrix incorporating type II collagen, aggrecan, and anionic proteoglycans [131]. After injection of male wild-type MSCs into

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female OI mice, 8% of cells grown from cartilage contained a Y-chromosome [62]. Injection of pre-labelled MSCs intraperitoneally into rats at the onset of collagen II arthritis resulted in the presence of labelled cells in joint cavities and sub-layers of proliferating synovial tissues, demonstrating their targeting ability. Furthermore, human MSCs injected intraperitoneally into fetal sheep contribute to articular cartilage chondrocytes, based on their appropriate location and characteristic morphology [7].

Fat

Human fat cells have been generated from MSCs in culture conditions [130] and when injected intraperitoneally into fetal sheep [7]. Conversely, adipose tissue has been viewed as a source of adult human stem cells with significant plasticity; yields of 200 000 undifferentiated cells per g are reported to have been purified from human adipose tissue harvested by liposuction [132,133].

Stem cell plasticity, transdifferentiation, or fusion confusion?

In the last month, concerns have been raised that the methods used to show that certain adult stem cells, particularly from the bone marrow and central nervous system, can jump lineage boundaries may be flawed: e.g. if reliance had been placed solely on the appearance of Y-chromosome-positive marrow-derived cells in a female recipient, or even if markers such as LacZ or GFP had been used. Two publications suggested that the development of apparently normal differentiated cells expressing a new marker might simply be due to the fusion of bone marrow cells with pre-existing differentiated cells in the host's organs. When bone marrow from GFP transgenic mice was cultured with embryonic stem (ES) cells, a very small proportion (2–11 hybrid clones per 10^6 marrow cells) of the bone marrow cells fused with ES cells and these cells could subsequently adopt some of the phenotypes typical of ES cell differentiation [134]. It should be noted that the frequency of hybrid cell formation was not greater in the haematopoietic fraction of bone marrow, particularly relevant since these are thought to be responsible for liver engraftment (see above). In separate experiments, a very low frequency of fusion (1 in 100 000) was described for cultures of CNS stem cells with ES cells and here the fusion cells were able to show multi-lineage potential when injected into blastocysts, most prominently into liver [135].

Cell fusion events were shown to be possible in tissue culture systems, not *in vivo* and the possibility that fusions (or heterokaryons) account for all instances of transdifferentiation or plasticity is at odds with a number of observations:

(1) When a few highly purified haematopoieteic stem cells from male Rosa26 bone marrow were used to rescue female mice deficient in FAH (fumaryl acetoacetate hydrolase) [78], there were clearly many discrete nodules of X-gal stain formed in the liver – unlikely if fusion events are rare. Further, the nodules had normal histology and were not teratoma-like (as seen after fusion events [134]).
(2) The thyroids of women with thyroid disease frequently contain male cells of presumed fetal origin, yet these were reported to possess just one X- and one Y-chromosome [136], rather than the XXXY predicted initially after fusion.
(3) Haematopoietic stem cells are abundant in cord blood and are found in peripheral blood especially after exercise: if fusion events were common and without disadvantage, we should all have large numbers of polyploid cells in many organs. This has not been reported outside the liver, where polyploidization does occur on a large scale, due to binucleate cells segregating on the same mitotic spindle.
(4) Therapeutic grafting of female patients with G-CSF-mobilized peripheral blood stem cells from male donors produced a variety of male cells including new hepatocytes in the liver, but all those shown had one X- and one Y-chromosome [137].

Until studies show that heterokaryon formation actually occurs when adult stem cells 'transdifferentiate' *in vivo*, then extrapolations from rare events involving cultured ES cells are premature.

Conclusions

There is now a large body of evidence indicating that organ-specific stem cells need not rely entirely on their own resources for maintenance and repair. In some circumstances, so far mostly associated with tissue damage, populations of stem cells normally resident outside the organ are able to contribute to the renewal of quite different lineages, even in tissues from a separate germ layer (Figure 2). Perhaps a key factor in the generation of self-renewing clones in the new tissues is the exposure to – and successful occupation of – niches emptied by damage, with the local environment of the niche defining the cell repertoire that will be produced [138].

Extraordinary claims require extraordinary proof and some have asked for a higher standard of evidence, requiring 'a clonal approach' [139] or demonstration of 'a robust, sustained multi-lineage engraftment and functional activity representative of multiple phenotypic characteristics of the converted cells to show that full conversion has occurred' [5]. These criteria, put simply, are needed because showing partial repopulation of an organ with cells that have come to resemble their neighbours is not the same as showing a functional competence as diverse and broad as that expected of the indigenous population. Yet this is what will be needed for tissue regeneration, and for gene

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therapy strategies relying on adult stem cell plasticity. We will need clonal expansion to yield all of the cell types normally produced, and only those, together with appropriate responses to the usual demands of growth, adaptation, and repair. So far, the experiments of Lagasse and co-workers [78,91] are closest to answering all criticisms, yet researchers working on other organs have shown transplanted bone marrow cells to effect a degree of rescue from, or transfer of, pathology [49,62,112,121].

A recent twist is that researchers who had reported that muscle satellite cells were haematopoietic now consider that their muscle-derived haematopoietic stem cells are likely derived from the presence of substantial numbers of haematopoietic stem cells normally resident in the muscle [140]. So, is it that there are in fact many multipotential cells distributed throughout the body? Something echoing the arrangements made in simpler organisms such as Platyhelminthes and planarians, where pluripotential neoblasts are distributed through the body competent to contribute to all maintenance and repair tasks [141,142]?

Given the considerable interest in therapeutic applications of stem cell plasticity, the truth should be worth waiting for.

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