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THE ROYAL SOCIETY

Developmental biology and the redirection or replacement of cells

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The aim of developmental biology is to understand how an egg converts itself into a complete organism through the processes of cell differentiation, morphogenesis and size regulation. The principles that have emerged over recent decades include the constancy of the genome in nearly all cells of an individual, the existence of stem cells in many organs and the overwhelming importance of signalling between cells for the determination of their fate. These and other characteristics of development are discussed here in relation to the prospect of achieving cell and tissue correction or replacement with the help of nuclear transplantation and signalling factors. Nuclear transplantation offers a one-step procedure for generating multipotent embryo cells from the cells of an adult tissue such as skin. It should be possible to proliferate the resulting cells as can be done for mouse embryonic stem cells. Embryo cells can be made to differentiate in many directions by exposing them to various agents or to different concentrations of a single factor such as the transforming growth factor β class signalling molecule activin. The possibility of a cancerous condition being acquired during these experimental manipulations can be guarded against by transfecting cells with a conditional suicide gene. Thus it may be possible to generate replacement cells or tissues from an adult human for transplantation back to the original donor, without the disadvantage of any genetic incompatibility.

Keywords: cloning; nuclear transplantaion; stem cells; genome; ricin; Prometheus

1. INTRODUCTION

Development is a remarkable process. In most kinds of animals, a single-celled, fertilized egg will convert itself, in two to three days, into a complete multicellular organism, with fully functional neuromuscular, circulatory and other systems, so that it is self-motile and can sense and respond to its environment. This extraordinary process of transformation takes place in the absence of any environmental instructions other than gravity. There is no increase in mass, since embryos cannot feed during this time, but only a conversion of yolk and other maternally inherited materials into differentiated cells. Mammals are an exception to this generalization, since their development is slow, and involves substantial growth due to the maternal environment.

The main components of development are cell differentiation that leads to the formation of highly specialized cells, morphogenesis that forms shapes and patterns, and growth that determines size. The long-term aim of developmental biology is to understand, in terms of populations of molecules, how these three processes are controlled. To fully understand development we need to know not only how an organism is formed, but also how the various tissues and organs are maintained, especially those that are continuously renewed throughout life.

In solving nearly all problems of basic science, there is also the prospect of useful applications that will provide human benefit. In the field of developmental biology, the prospect of cell correction or replacement is of enormous potential benefit to mankind. As we get older, the normal processes of cell renewal, for example in skin, intestine and blood, may become uncontrolled, leading to cancer, and in our advanced years, most of our tissues cease to function efficiently. If we could fully understand how embryonic cells follow their normal pathway to specialization and controlled replacement, we should be able to correct cells that have deviated from normality or from efficient function, or to train spare embryonic cells to provide replacements for older cells that have exceeded their useful lifetime.

The purpose of this article is to summarize briefly some of the principles that we have come to understand about development, to indicate the kind of information that we can realistically hope to acquire in the foreseeable future, and lastly to explore the future prospects for cell and tissue redirection and replacement.

2. PRESENT UNDERSTANDING

(a) *Normal development*

In most animals, development consists of an early phase of rapid cell division for a few hours after fertilization, a phase of complex cell movements and rearrangements, and a phase of cell specialization. Thus the main 'body plan' is fully formed within a few days of fertilization. After this, feeding commences, growth takes place and cell replacement in some tissues takes place for the rest of the organism's life. In mammals, the early stages take place on a much slower time-scale, and growth comes predominantly from the mother rather than from yolk reserves of the egg. Nevertheless, the overall construction of a mouse embryo

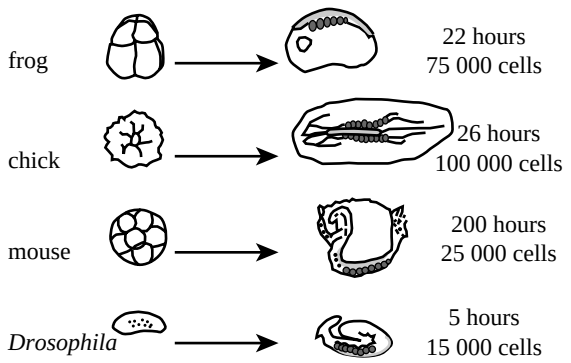

Figure 1. Similarity of vertebrate embryos. Although the eggs and earliest stages of development look very different in the frog, chick, and mouse, the early axis and somite stages are morphologically similar. Left, embryo at eight-cell or eight-nucleus stage. Right, embryos at somite stage. Mouse (and human) development is very slow compared to non-mammalian vertebrates.

(or human embryo, which is very similar) eight days after fertilization resembles that of a frog at 22 h or of a chick embryo at 26 h after fertilization (figure 1). For general accounts of development, see Gilbert (1997), Davidson (1986) and Wolpert (1998). These books also provide literature sources for many of the unreferenced statements in this article.

The developmental mechanisms that determine cell fate, and that are most relevant to the present discussion, concern the constancy of the genome, the existence of stem cells and their ability to generate daughter cells of different types, and the importance of transcriptional control in determining cell fate.

(b) *The constancy of the genome*

Largely as a result of nuclear transplantation experiments in amphibia (review by Gurdon 1986) and more recently in mammals (Wilmut *et al*. 1997; Wakayama *et al*. 1998), it is now recognized that the genome does not undergo any stable changes in the course of development (figure 2). An exception to this rule is provided by lymphocytes, which undergo variable, diversity and joining (VDJ) gene recombination for the formation of antibodies and antigen receptors. From the point of view of the redirection of cells, as well as for understanding development, the constancy of the genome is a key principle. At least in theory, it must be possible to transform any one cell type into any other that shares the same genome.

(c) *Cell lineage, stem cells and regeneration*

In some animals, notably in nematodes, there is an invariant cell lineage in early development, such that each embryo cell always gives rise to the same cells of an adult, a characteristic that greatly impressed early embryologists (e.g. Wilson 1896). However, the significance of this has become clarified in recent years by our appreciation of two principles. One is that, in most animals, especially the vertebrates, there is no simple lineage relationship between the position of a particular cell in the embryo and the fate of its direct descendants. Furthermore, in all animal embryos, including nematodes, the killing or removal of

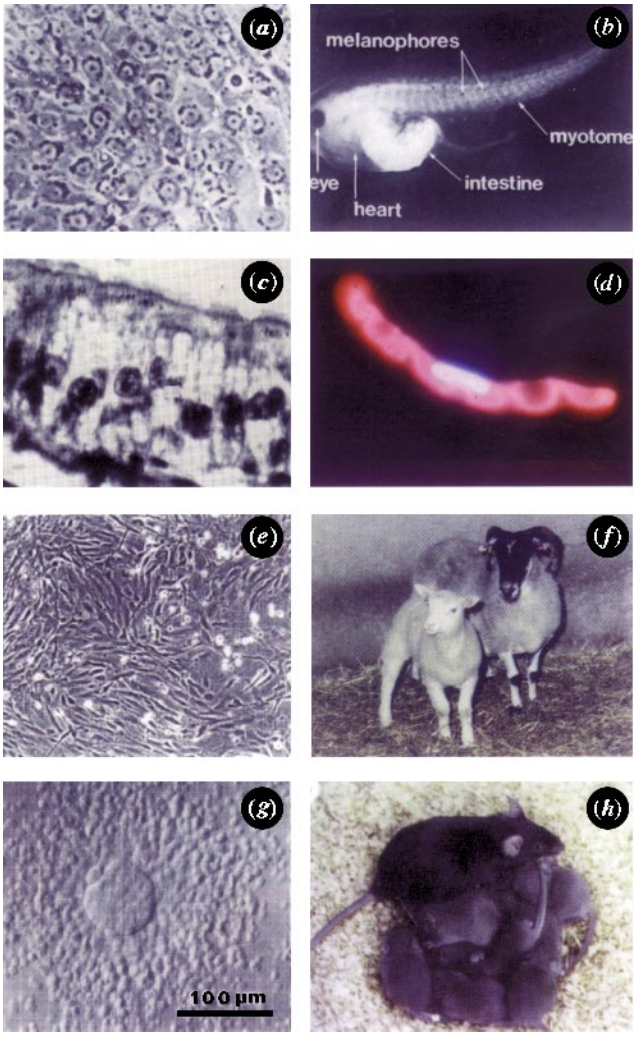

Figure 2. Constancy and totipotency of the genome. Amphibia: (*a*) cultured cells grown out from an explant of the foot-web of an adult frog, showing the one-nucleolated genetic marker; (*b*) swimming tadpole obtained by transplanting a nucleus from a cultured skin cell, as shown in (*a*); (*c*) intestinal epithelium of a feeding tadpole from whose transplanted nuclei fertile adult frogs were obtained; the striated or brush border of the epithelial cells can be seen; (*d*) single differentiated muscle cell from a swimming tadpole, from the nuclei of which tadpoles like that shown in (*b*) were obtained. Original references to amphibian nuclear transfer work are cited in the review by Gurdon (1986). Sheep: (*e*) cultured cells grown from a mammary gland; (*f*) a nuclear-transplant lamb and its foster mother (black head) obtained from the transplanted nucleus of a cell such as those shown in (*e*); Wilmut *et al*. 1997. Mouse: (*g*) an oocyte and surrounding cumulus cells; (*h*) a mouse obtained by transplanting a nucleus from a cumulus cell, and her offspring; Wakayama *et al*. 1998. (*e*,*f*) Reprinted with permission from Wilmut *el al*., *Nature*. © 1997 Macmillan Magazines Ltd., (*g*,*h*) Reprinted with permission from Wakayama *et al*., *Nature*. © 1998 Macmillan Magazines Ltd.

one cell will commonly result in a compensatory change of fate in neighbouring cells. Therefore the differentiation of an embryonic cell is determined primarily by its neighbours not by its ancestry. The constancy of cell lineage in nematodes and other invertebrates seems therefore to be best explained by the constancy of signals emitted by neighbouring cells and not by a cell's history.

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The second important principle is that, throughout life, some tissues are subject to continual replacement, and these include blood cells, skin cells and intestinal epithelium cells among others. These cells have a relatively short life, for example 60 days for erythrocytes, compared to non-renewing tissues such as the lens body, cephalic neurons and others, which last the whole of a lifetime—up to about 100 years in humans. Cell replacement in renewing tissues is due to the presence of so-called stem cells; these are unspecialized cells that are able to divide and to yield daughter cells that can either differentiate or proliferate to make more stem cells. It is believed that stem cells exist with various degrees of commitment, some being able to form only a limited range of terminal cell types, while other 'early' stem cells have a much wider choice of specialized cell types that they can generate. A stem cell seems to have the option of generating daughter cells that are proliferative (more stem cells) or differentiative (progenitors of specialized cells) or one of each kind. Somatic stem cells have been investigated most fully in mammalian blood and neural tissue, but clearly exist in mammalian gut and skin (reviews by Morrison *et al.* 1994, 1997). One of the best known examples is that of haemopoietic stem cells, some of which are recognized by the cell surface antigen CD34. However, for most tissues, the stem cells that are presumed to exist do not have markers by which they can be recognized and are therefore not well characterized or even, in most cases, identified. When such cells are identified and able to be isolated as a result of future work, they should be of great value for tissue replacement.

The ultimate form of tissue replacement is seen in regeneration. According to ancient Greek literature, Prometheus, having stolen the secret of fire from Zeus, was punished by being chained to a rock, where his liver was eaten daily by a long-winged eagle; his 'immortal liver' regenerated overnight, to replace the amount eaten by the eagle (Hesiod 735 BC; Michalopoulos & DeFrances 1997). More recently, and more reliably, it has been established (Michalopoulos & DeFrances 1997) that a rat can regenerate its liver after 12 sequential hepatectomies, and that a single hepatocyte can divide at least 34 times to give over 10^{10} cells (50 times greater than a normal rat liver). Within the vertebrates, newts have exceptional powers of regeneration (Brockes 1997) since they can regenerate a whole limb (figure 3), the lens of an eye and other organs. Other special cases of regeneration exist. For example, the antlers of deer. However, for most tissues and organs, humans and other vertebrates have very limited powers of regeneration; missing limbs and eyes can not be replaced. In those animals where some regeneration does occur, transdifferentiation, that is a switch from one cell type to another, occasionally takes place (Okada 1991). Much more commonly, it is believed that stem or progenitor reserve cells are stimulated to replace a correct amount of missing material of their own type, as in compensatory growth of the liver or kidney. The mechanisms by which organs are regenerated and maintained to the correct size have recently been reviewed (Conlon & Raff 1999). A principle involved seems to be that cells recognize the ratio of molecules that promote or inhibit cell division; the inhibitors would be restricted to an organ such as the liver, whereas the promoter molecules would be distributed throughout the organism, for example in the blood. It is simplest to suppose that promoter molecules would be synthesized throughout the body, whereas the inhibitory molecules would be synthesized only by an organ capable of regeneration; when the organ has regenerated to its full size, the growth inhibitors would predominate over or sequester growth promoters.

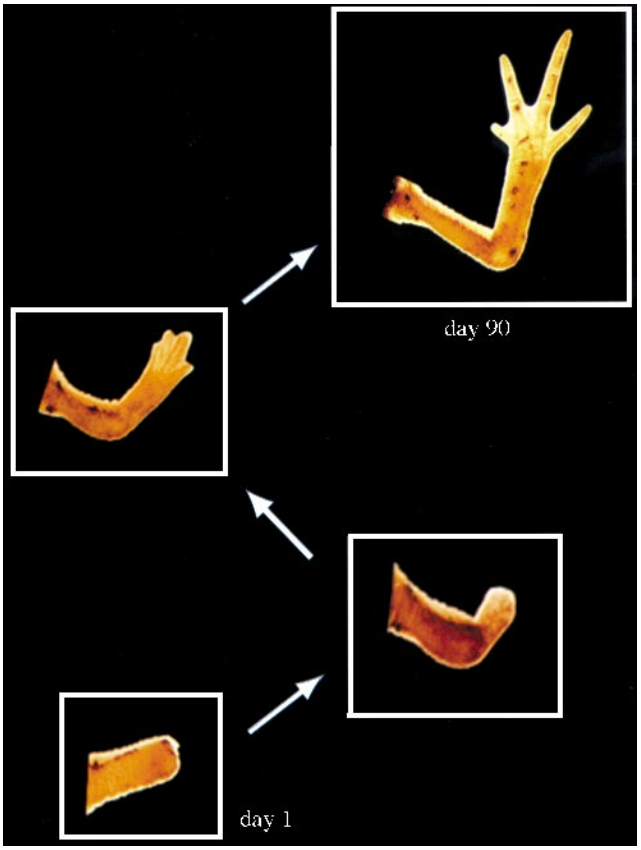

Figure 3. Regeneration of the forelimb of an adult newt (*Notophthalmus viridescens*). Courtesy of Dr A. Kumar and Dr J. Brockes.

(d) *Control of gene expression*

Cell differentiation and cell fate determination depend on differential gene expression; this causes the protein content of the various cell types formed during development to become increasingly divergent. We know that nearly all types of cells have the same complete genome, and yet the great majority of the proteins in most differentiated cells are encoded by less than 10% of their genes. By far the greatest contribution to this effect is provided by differential transcription. Thus a haemoglobin gene during the formation of red blood cells is transcribed at least 1000 times more frequently than the same gene in a skin or brain cell, where it is not transcribed at a detectable rate. Although there are clear examples of translational control, i.e. the same kind of mRNA being translated much more frequently in one cell type compared to another (Gray & Wickens 1998), this makes a rather minor contribution to overall cell-type differences compared to transcriptional control. The same applies to the post-translational modification of proteins. Furthermore, mRNA characteristic of one cell type, such

as mRNA encoding globin, is efficiently and immediately translated when introduced into cells of an entirely different type, such as muscle. Therefore a change in gene transcription in a cell should lead to a corresponding change in the differentiation of that cell.

By what mechanism is differential transcription achieved? It now seems clear that this depends very largely on the combination of transcription factors with which a cell is endowed. By binding to particular sites on promoter or enhancer DNA sequences near a gene, transcription factors regulate the extent to which a gene is transcribed. The overwhelmingly predominant nuclear activity in early embryos is the synthesis of transcription factors and the exchange of signals between cells. It is believed that over half of all genes that are activated at the beginning of animal development encode transcription factors. Of the remainder, a high proportion code for secreted signalling factors or for components of the pathways that transduce the signal from cell surface receptors to the nucleus. In species where early events have been analysed, we see a reciprocating cascade of events. Cells in one region of an embryo emit signalling molecules, which induce in other cells the expression of genes that encode new transcription factors or other signalling molecules. An increasingly complex range of genes becomes active, eventually defining cell and tissue types in various parts of an organism.

It is interesting to ask how the whole process starts, since development begins with a single cell, which cannot therefore engage in intercellular interactions. In the great majority of animals, development starts with the asymmetrical distribution of determinant molecules in the fertilized egg, so that daughter cells differ in their content of these molecules. This enables their progeny to signal to each other, and thus to initiate the signalling and transcription factor cascade. At some later stages of development, asymmetrical cell division is again important in generating or amplifying cell-type differences (Jan & Jan 1998).

An important feature of signalling mechanisms in development is that a limited variety of signalling factors can activate a surprisingly wide range of genes. In part this is due to the phenomenon of competence, by which cells gain and lose the ability to respond to a particular signalling factor; even the same cell may change competence within a few hours, losing the capacity to respond to one signalling factor while gaining competence to respond to another. Also in part, the wide range of effects attributable to a signalling factor is due to the ability of cells to make concentration-related responses. The same cell can express at least five different genes according to the concentration of a factor to which it is exposed (Huang *et al*. 1997). Although the molecular basis of competence and concentration-dependent response is not yet understood, both processes show that it is possible to generate a range of cell types by supplying appropriate signals or different concentrations of one signal to a single kind of embryonic cell.

3. FUTURE UNDERSTANDING

(a) *Gene transcription*

Automated methods of sequencing genomes are well in hand, and a representative genome will soon be sequenced for humans and for several of the most widely used research species. This will surely give a useful guide to the function of all genes in a genome. Even more valuable for the analysis of development will be mircoarray and proteomics technologies, by which large numbers, and maybe all, of the more abundantly expressed gene products can be quantitated by semi-automated methods. This will eventually provide for most genes a measure of the amount of RNA and protein present in cells. Looking further ahead, we will need to have this information for individual cells, rather than average values for cell populations. *In situ* hybridization of cDNA or cRNA probes to whole-mount or sectioned material can show single-cell localization of transcripts. The same can be said of antibodies for proteins, and this is much more important because, for nearly all genes, it is proteins, not mRNA that have cell fate-determining activity.

Sensitivity with current procedures can be achieved to a level of about 100 molecules per cell, but only for those few genes for which excellent probes or antibodies exist. One hundred molecules per cell is a tiny fraction of the typical approximately 10^{10} proteins per cell. This sensitivity of recognition works out at a concentration of about 1 pMol for 100 molecules of a protein such as a transcription factor that is concentrated in the nucleus, and this is probably well below the concentration at which most proteins concerned with gene regulation or cell-type differentiation have an effect. This is especially true of regulatory molecules that determine cell fate, i.e. ones without enzymatic activity, as judged by the following line of argument. A protein that binds specifically to a DNA sequence, such as the promoter of a single copy gene, will bind unspecifically to many other DNA sequences with an approximately 10^6-fold lower affinity. Therefore only by providing a cell with about 1000 molecules of this protein will enough be available to have a high chance of binding to the specific promoter sequence, as well as to a random assortment of other sequences. It is true that the cooperative binding of two or more proteins can increase specific versus unspecific binding. However, those vertebrate regulatory proteins and RNAs so far known to act specifically are present in cells at much more than 10^3 molecules per cell. Therefore we can expect in future to know the concentration of RNAs and proteins in each cell with a precision much greater than the biologically relevant level.

It is becoming apparent that cell function is surprisingly sensitive to small changes in concentration. For example, a threefold change in the concentration of the transforming growth factor (TGF) β class signalling molecule activin will cause cells to switch expression from one regulatory gene to another, embarking on very different cell fate pathways, for example from ventral mesodermal (blood) to dorsal mesodermal (backbone and nervous system) differentiation (Green *et al*. 1992; figure 4 and table 1). Although a twofold difference in the amount of a gene product usually makes no difference, since heterozygotes for most mutations are normal, even this is not always so, as for genes that show haplo-insufficiency. Thus one challenge for the future of gene regulation, and hence of cell differentiation, seems to lie in finding ways of determining and controlling small changes in concentrations of regulatory molecules and

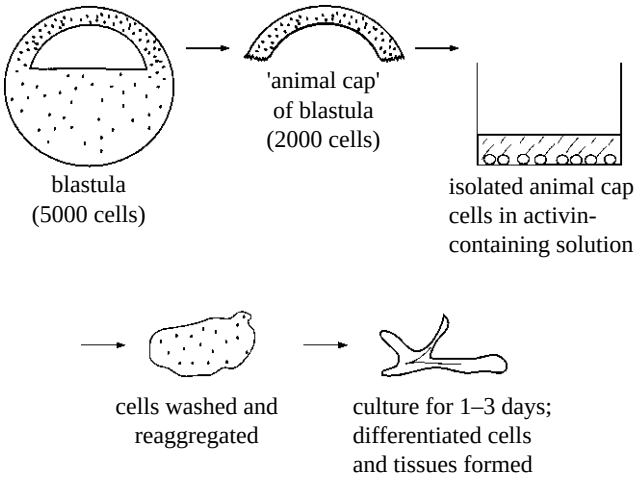

Figure 4. Differentiation of embryo cells by exposure to the TGFβ class secreted factor activin. *Xenopus* blastula cells from an 'animal cap' can be dissociated, briefly treated with different concentrations of activin in their medium, reaggregated and then cultured or transplanted to hosts. According to the concentration of activin in the medium, entirely different cell types are generated. Based on the results of Green *et al*. (1992).

not in needing to quantitate very small absolute numbers of these molecules per cell.

A full understanding of development will probably require, in addition to a knowledge of the concentration of RNAs and proteins per cell, a knowledge of the half-life of key molecules; some RNAs and transcription factor proteins have half-lives of less than 1 h, while structural proteins, such as lens crystallins, probably last for 100 years or so. The affinity and hence duration of binding between molecules will need to be known for key inter-molecular interactions. Lastly, the movement of molecules within and between cells will be of great importance. We should assume that all of this information will become available within a decade or so.

(b) *Developmental phenomena*

There are certain phenomena in development that do not appear, at present, to have the kind of explanation to which we are accustomed in cell and molecular biology. These are of special interest and curiosity and are relevant to the formation of replacement cells and tissues.

One of these is the problem of how a radially symmetrical cell such as an egg can form embryos with structures, such as the heart, consistently located on the left or right side. In most animals, the anterior–posterior and dorsal–ventral axes are set at or before fertilization, but the left–right axis is certainly not preformed in the eggs of those species where the position of the sperm entry point determines one of the first two axes. Although many early genes are now known to be expressed on one or other side of an embryo, it is still quite obscure how the first molecular differences are established on one side or the other of a bilaterally symmetrical egg.

A second developmental problem concerns the basis of competence. There are both temporal and regional constraints on the ability of cells to respond to extra-cellular signals. For example, amphibian blastula cells have the ability to respond to mesoderm-inducing factors of the TGFβ class, but lose this ability at the early gastrula stage, when they acquire the ability to respond to neural inducing factors. Changing competence enables embryos to make use of the same signalling factor for quite different effects. The molecular basis of competence is not known.

Table 1. *Small changes in the concentration of the same signalling factor generate different cell types*

activin concentration (pMol)	early gene activation	eventual cell-type differentiation
0	epidermal; keratin	epidermis; cilia
1	epidermal; keratin	epidermis; cilia
3	ventral mesodermal; Xbra; zygotic Veg T	blood; kidney
10	lateral mesodermal; XMyo D: XMyf 5	muscle
20	dorsal mesodermal; Xgsc; Xeomes	head mesoderm; brain induction
30+	endodermal; Xsox17; endodermin	intestine; pancreas; liver

Lastly, the timing of developmental changes is under the control of a clock, by a mechanism so far unidentified. A signalling agent, such as a member of the TGF family, can cause embryo cells to embark on different fates; it does not matter whether such a factor is administered at an early or late time, but the gene response made by cells will always take place at a particular developmental age. To be able to fully control cell differentiation *in vitro* will require knowledge of developmental phenomena of these kinds, and this is unlikely to emerge from current advances in genome sequencing, automated RNA and protein arrays, etc.

(c) *Organ construction*

Almost all organs and tissues in the body consist of mixtures of overtly different cell types, usually of different germ-layer origin. Furthermore, the cells of different kinds have a clearly defined spatial relationship to each other. The liver, for example, consists of at least five different cell types arranged in plates. We are a long way from understanding how these complex patterns and cell assemblies are formed. We know that external forces can influence the shape of a bone, and that the differentiation as well as behaviour of cells is greatly affected by the extracellular matrix in which they lie. However, some success has already been achieved, in the academic and biotech company worlds, in growing tissues such as skin and bone in culture. Furthermore, it seems likely that one cell type within a complex organ, such as parenchymal cells of the liver, can be made to carry out many of their principal functions in the absence of other cell types.

4. PROSPECTS FOR CELL CORRECTION AND REPLACEMENT

Subjects like astronomy and evolution hold a fascination for the human mind, even though, if everything were understood, it hardly seems likely that either field of study would be useful for our health or economy. How an

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egg or seed converts itself into an animal or plant is a truly remarkable phenomenon, and has an obvious scientific interest. But, in addition, an understanding of these processes seems certain to be useful to those who have no knowledge of, or even interest in, science. This is because this knowledge would open the way to cell correction and cell replacement of our tissues and organs as these become non-functional due to disease, age or damage.

(a) *Cancer cells*

The most obvious need for cell correction applies to cancer. To be able to prevent the continuing proliferation of cancer cells would, in most cases, remove their lethal effect, even if they could not be made to resume their normal function. If we could understand the normal processes by which embryonic or stem cells generate non-dividing differentiated cells, it should be possible to impose such processes on cancer cells. Even if deviant cells are genetically impaired, perhaps through possession of a constitutively active growth factor receptor, this would not necessarily preclude correction of their phenotypes by non-genetic means. For example, the correct concentration of a suitable combination of signalling factors might be able to force cancer cells into a differentiation pathway. Interference with signalling pathways required for proliferation is another obvious direction of research under current intense investigation. For the present purpose, we need only point out that, as the process of cell differentiation becomes understood by work in the field of developmental biology, the over-expression of differentiation pathways may be useful in nullifying the harmful effect of cancer cells, even if they cannot be retrained into useful cells.

(b) *Cell replacement with stem cells*

The cell and tissue replacement that takes place continuously in normal life implies the existence of reserve or stem cells in many of our tissues. What are the prospects for using these as a source of cells for expansion in culture and transplantation to the donor? Two ideas prevail. One is to identify and proliferate the desired type of stem cell. This approach has been particularly successful for haemopoiesis (figure 5), and is already in clinical use especially after chemotherapy. Early haemopoietic stem cells can be selectively amplified in culture and made to differentiate into eosinophils and neutrophils by granulocyte macrophage-colony stimulating factor, or into antibody-producing plasma cells and activated T cells by interleukins (figure 5). Haemopoietic stem cells are obtained from bone marrow, and are already committed to be able to form only various types of blood cells. For most other tissues or organs, stem cells have not yet been identified, and it is not therefore known whether such cells, if they exist, can be increased in culture.

The other idea is to make use of embryonic stem (ES) cells (figure 6). These cells, first grown from the pre-implantation inner cell mass of mouse embryos, can be maintained in culture indefinitely in an undifferentiated state by growth on the surface of embryonic fibroblasts or in complete medium containing leukaemia inhibitory factor (Evans & Kaufman 1981; Martin 1981). At any time, ES cells can be made to differentiate in culture by removing leukaemia inhibitory factor or preventing substrate attachment so as to promote cell–cell contact in aggregates called embryonic bodies. Differentiation can be further promoted by adding such agents as DMSO (a chemical not found in living tissue), retinoic acid (related to vitamin A), activin, interleukins, etc., to the medium. Differentiation in the directions of neural tissue, haemopoietic tissue or cardiac muscle is easily achieved. Differentiation in other particular directions can be promoted by overexpressing appropriate genes, such as MyoD to promote skeletal muscle. A wide range of cell types can be generated if ES cells are injected into immunocompromised mice (Keller 1995; O'Shea 1999). Recently two reports have appeared of human ES cells and these have extensive proliferative potential, as well as the capacity to form many different cell types though not, as yet, to contribute to the germ-line (Thomson *et al*. 1998; Shamblott *et al*. 1998). To be useful for human cell replacement (Keller & Snodgrass 1999), ES cells or their clonal descendants would have to be transplanted to immunologically incompetent hosts, or to immunologically protected sites. Or ways would have to be found of generating such cells from an adult in order to transplant them back to the original donor.

(c) *Nuclear transplantation*

An alternative route to the provision of replacement cells is to use nuclear transplantation, or conceivably cell fusion, to generate embryonic cells, starting with the nucleus of an adult cell.

As mentioned above, cells can be grown out from explants of nearly all adult organs in culture. The cells that emerge in the course of a few days from a small piece of tissue are generally of fiboblastic morphology. The nuclei of adult cells have been used successfully for transplantation to enucleated *Xenopus* eggs to generate swimming larvae containing all major somatic cell types in a functional state (table 2; Laskey & Gurdon 1970). The same is true for sheep and mice (Wilmut *et al*. 1997; Wakayama *et al*. 1998). It should therefore be possible to derive 'embryonic' cells from any adult organ by nuclear transplantation—which is, in effect, a way of using the reprogramming activity of egg cytoplasm to reset the nucleus of an adult cell back to an embryonic state. Such embryonic cells should be responsive to the same treatment with secreted ('growth') factors as can be applied to normal embryonic cells, so as to generate specialized cells of a desired kind. This would be a way of generating embryonic cells from adult tissue. It could be suggested that the use of adult somatic cells for nuclear transplantation would run the risk of propagating mutations, which probably accumulate with age, and which might initiate cancers. However, the rejuvenated embryonic cells derived by nuclear transplantation would need to be grown in culture, like embryonic stem cells, before and during treatment with 'differentiation' factors, and could be checked and selected for normality at this stage. The use of nuclear transplantation could provide a single-step reversal of adult cells to an embryonic state, and this would be far simpler than trying to reverse the normal process of differentiation step by step.

The formation of hetero- or syn-karyons by fusing an adult tissue cell with an actively dividing cultured cell

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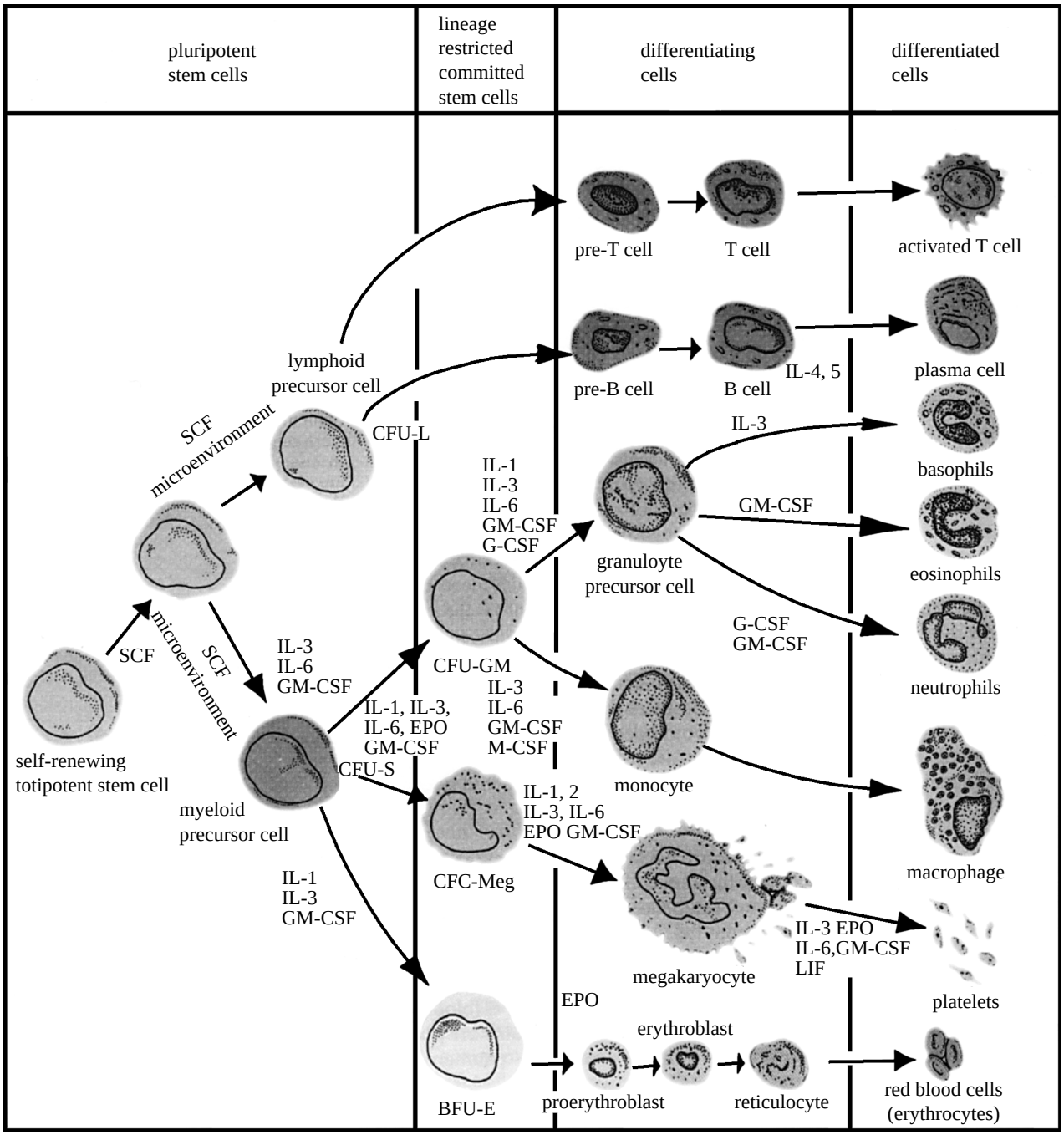

Figure 5. Stem cells and haemopoiesis. Mammalian bone marrow cells include different states of stem cells, some pluripotent and others more lineage restricted. By the addition of purified growth and differentiation factors to culture media, stem cells can be induced to proliferate or to generate various types of circulating blood cells. From Gilbert (1997).

might provide another route to the rejuvenation of gene activity. The appropriate choice of fusion partners can yield cells with the desired characteristics. The classic example of this is the fusion between a proliferating myeloma cell and an antibody-synthesizing B cell to form a fusion hybrid that continues to proliferate as well as to secrete monoclonal antibodies. However, the proliferative activity imposed on an adult cell is provided, in this case, by a transformed and therefore potentially malignant cancer cell.

The potential value of nuclear transplantation for creating embryonic cells from adult cells seems enormous. At present there is one constraint. This is that the great majority of eggs receiving a nucleus from the cell of an adult develop very abnormally; the proportion that form complete larvae or foetuses is no more than 1% in frogs and in mammals. Those nuclear transplants that do not develop normally might nevertheless provide a source of embryonic cells that can be differentiated by exposure to appropriate concentrations and combinations of factors and the most normal cells can be amplified by serial nuclear transplantation. The reason for abnormal development, at least in amphibia, appears to be the failure to complete chromosome replication before the egg divides, and future research will certainly investigate ways of improving this, for example by decondensing nuclei

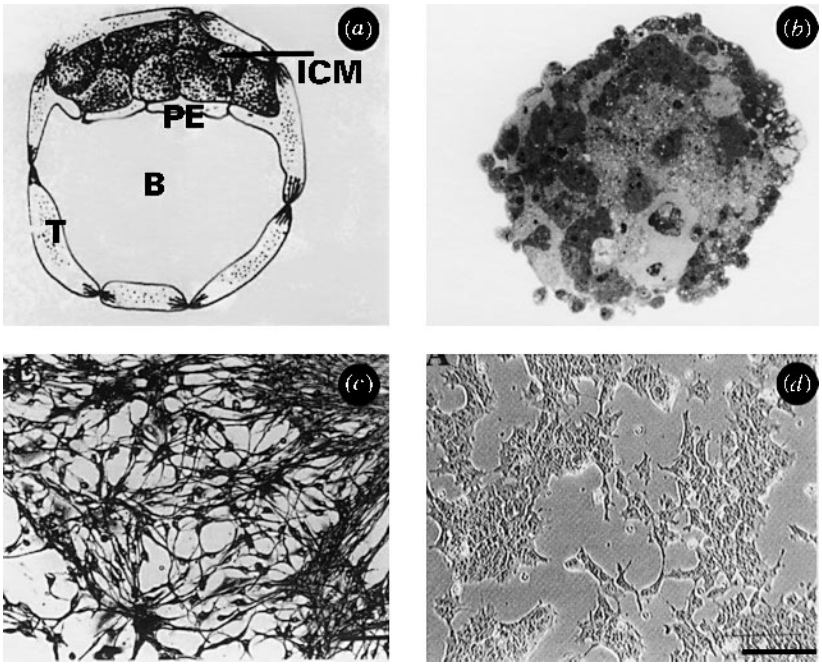

Figure 6. Mouse embryonic stem cells and their differentiation. (*a*) A four-day mouse embryo blastocyst; cells of the inner cell mass (ICM) are used to create a permanent line of embryonic stem (ES) cells that can be proliferated indefinitely in culture. PE, primitive endoderm; B, blastocoel; T, trophoblast. (*b*) When unattached to a substrate, ES cells make aggregates (embryoid bodies) containing many cell types in a disorganized arrangement. (*c*,*d*) ES cells can be made to differentiate into nerve cells (*c*), or muscle cells (*d*) by addition of appropriate factors to the medium, or by overexpression of cell type-specific transcription factors. From O'Shea (1999), *Anatomical Record*. © 1999 Wiley–Liss, Inc. Reprinted by permission of Wiley–Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

Table 2. *Reprogramming of gene expression by nuclear transplantation in amphibia*

(A single nucleus is transplanted from the donor cell type indicated to an enucleated egg which is grown through embryonic stages. Such embryos are tested for DNA, RNA or single gene transcripts at a late blastula or early gastrula stage (about 8 h) or at an early tadpole stage (about 24 h). Original work cited in Gurdon (1986).)

nuclear donor cell type	type of nuclear activity	level of nuclear activity			conclusion
		donor	early embryo	late embryo	
adult skin cells	DNA synthesis	off	on (fast)	on (slow)	egg activates DNA synthesis
embryo endoderm	ribosomal RNA synthesis	on	off	on	egg reversibly inhibits ribosomal RNA synthesis
tadpole muscle	muscle actin transcription	on	off	on in muscle	egg reversibly inhibits muscle genes
cultured cell line	early genes Xgsc, Xwnt8, Mix1, Xbra	all off	mostly on	off or low, as in normal embryos	egg reversibly reactivates most early embryo genes

before nuclear transfer. The prospects for eventual success are of course favourable, in view of the fundamental principle of the constancy of the genome.

(d) *Combination with gene therapy*

As the sequencing of the human genome reaches completion, and as the identification of gene function in relation to human disease progresses, there will be increasing opportunities to combine gene therapy with cell transplantation. Procedures are well developed for transfecting cloned genes into cultured mammalian cells and for selecting the desired transformants. Therefore embryonic or adult stem cells, whether prepared from foetal or adult tissues, and whether grown directly from explanted tissues or prepared by nuclear transplantation, can be transfected with cloned DNA, and selectable markers can be used to grow up suitably modified cells. The coupling of gene transformation with cell transplants can benefit human health in two ways.

One is to take cells from an individual who is deficient for a gene, transfect the cloned gene into cells cultured from this individual, and then supply the cells back to the individual. This is the traditional route for gene therapy. It can work well for cases in which a few cells with functional genes can compensate for the failure of a much greater number of host cells. For example, a few cells expressing the depurinating enzyme adenosine deaminase (ADA) can compensate for the inability of most cells to do so, because one enzyme molecule can process a lot of substrate in the circulating blood. It is a great deal harder to make this process work well when normal tissue function requires that all or most cells should have the necessary gene activity, as for example in cystic fibrosis or muscular dystrophy.

The other way in which the coupling of gene transfer with cell transplantation could be useful is to provide a safeguard against the potential hazard of transplanted cells becoming cancerous. It is certainly possible that the combination of nuclear transfer with treatment of cells by growth and differentiation factors could lead to the formation of cancers in recipient hosts, especially after these cells have been grown and amplified in culture.

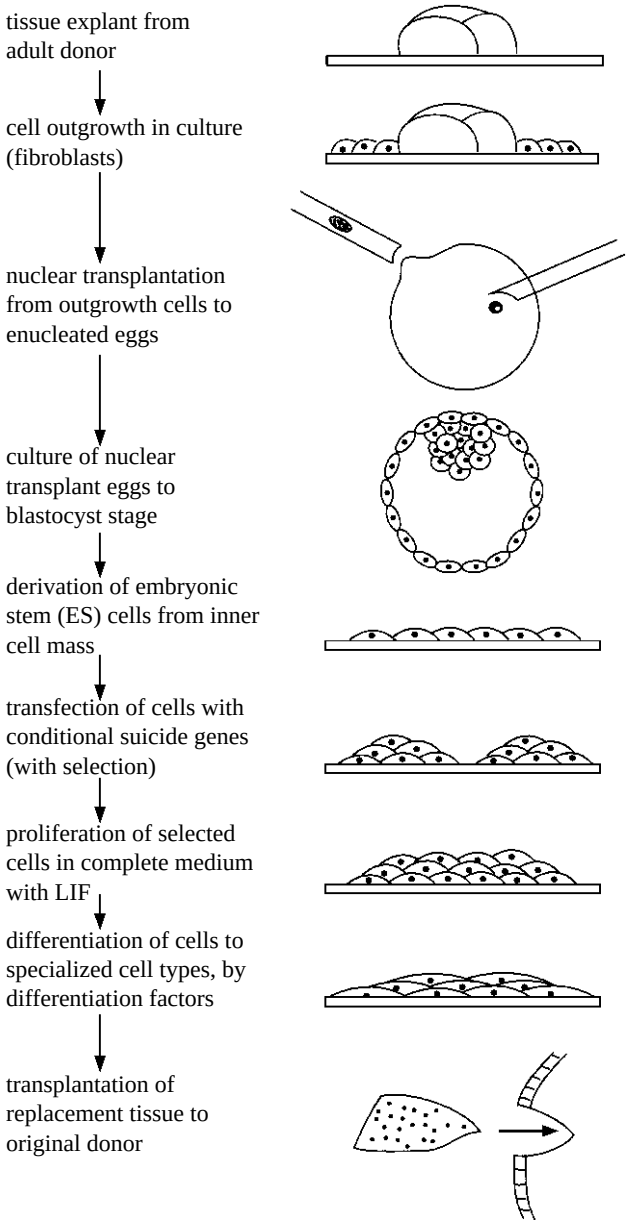

Figure 7. Summary diagram. This illustrates a route by which a small piece of tissue, such as skin from an adult mouse (or human), might be used to generate cells or tissues for replacement. The regimen involves nuclear transplantation to generate embryonic cells directly from adult tissue, and treatment with a combination of differentiation factors (or with different concentrations of one factor) to generate different cell types and tissues *in vitro*. Finally, the cells or tissues are transplanted back to the original donor, thereby avoiding the need for immunosuppression.

However, the danger of using cells of this kind for transplantation to humans should be avoidable by introducing into such cells a gene that sensitizes them to conditional killing. An example of a suicide gene is ricin, a dimeric protein derived from the castor oil plant *Ricinus*. The B chain is needed for cell penetration, and the A chain, which cannot spread from cell to cell, has an immensely powerful cell killing effect by a ribonuclease activity on ribosomes, thereby preventing protein synthesis. The efficacy of trace amounts of ricin was vividly demonstrated in 1978 when a Bulgarian dissident, Georgi Markov, died a few days after being touched in the street with a ricin-tipped umbrella (Andrew & Gordievsky 1991). It might be possible to place a ricin A-chain gene under the control of a non-mammalian hormone response element, such as that of the insect moulting hormone ecdysone, and transfect that construct into adult-derived embryonic cells for transplantation. In the event of some of the transplanted cells becoming malignant, a dose of ecdysone (which, incidentally, is not present in adult mosquitoes) would eliminate the introduced cells.

An even better prospect for the future would be to use a mechanism in which cells can divide for only a limited number of divisions. If cells to be transplanted could be provided with a limited life span of, say, 15 divisions (30 000 cells), any one cell would never form a tumour of a size to matter, but an implant of 10^8 cells (less than 1 ml) would generate over 10^{12} cells before ceasing to divide, and this should suffice for most cell replacement needs. The progressive reduction in telomeres, in the absence of telomerase, provides one example of how cells can have a limited replication potential.

5. CONCLUSIONS

The aim of this article has been to show that cell correction and replacement is not only theoretically possible, due to genetic constancy in somatic cells, but also that the various technologies required for its successful execution already exist, at least in a rudimentary form (figure 7). Most importantly these include (i) the existence of embryonic as well as adult stem cells that can be made to proliferate or differentiate in culture; (ii) the ability of appropriate concentrations of purified factors to cause major switches in the direction of differentiation of embryonic cells; and (iii) the ability to reverse the differentiation of adult cells back to an embryonic state by nuclear transplantation. Substantial further advances in our understanding of development, at the levels of both cell differentiation and morphogenesis, will be needed to create functional tissues or organs from embryonic cells, but there is every reason to suppose that such advances will take place within the next decade or so.

There is a tendency among some members of society to see scientific advances as threats, interference with nature, playing God, etc., and to try to arrest by legislation the progress of science. It is hard to imagine that the redirection or replacement of cells as proposed here could have harmful consequences. The worst outcome would appear to be the formation of cancer cells, but it should be possible to introduce genetic safeguards by which transplanted cells could be killed if necessary. It is important to appreciate that there would be no danger in two respects where concern might be felt. One is that the genetic and cell modifications proposed would not affect the germ-line and would not therefore be inherited. The transplanted cells would last only as long as the life span of the recipient. The other potential concern is that life might be thought to be potentially infinite through continuous replacement of the whole body. In practice, this is unrealistic, most particularly in respect of the brain. Even if ways should eventually be found of replacing neurons, the learnt experience of the brain acquired during a lifetime would presumably not be regenerated,

and the same individual would not be reformed. In summary, it seems clear that the benefit that can be expected from the use of redirected or replaced cells will far outweigh any foreseeable disadvantages. This is why the further analysis of development has so much to offer for the benefit of mankind.

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