Nuclear reprogramming in cell-free extracts

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Methods for directly turning a somatic cell type into another type (a process referred to as transdifferentiation) would be beneficial for producing replacement cells for therapeutic applications. Adult stem cells have been shown to display a broader differentiation potential than anticipated and may contribute to tissues other than those in which they reside. In addition, novel transdifferentiation strategies are being developed. I report recent results on the functional reprogramming of a somatic cell using a nuclear and cytoplasmic extract derived from another somatic cell type. The reprogramming of 293T fibroblasts in an extract from T cells is evidenced by nuclear uptake and the assembly of transcription factors, induction of activity of a chromatin remodelling complex, changes in chromatin composition and activation of lymphoid cell-specific genes. The reprogrammed cells express T-cell-specific surface molecules and a complex regulatory function. Reprogramming cells in cell-free extracts may create possibilities for producing replacement cells for therapeutic applications. The system may also constitute a powerful tool to examine the mechanisms of nuclear reprogramming, at least as they occur *in vitro*.

Keywords: cell extract; chromatin; cell signalling; nuclear reprogramming; transdifferentiation

1. INTRODUCTION

Epigenetic modifications allow differentiated cells to perpetuate the molecular memory needed for the cells to retain their identity. These modifications are heritable. Nevertheless, they may also be experimentally reverted as evidenced, for example, in the cloning of animals by transplantation of somatic cell nuclei into oocytes or in cell hybridization studies. Yet, reversion of the somatic cell nucleus to totipotency by nuclear transplantation is not an efficient process, as success is typically achieved in only one out of a few hundred attempts. Methods for directly and efficiently reprogramming a somatic cell into another somatic cell type (a process referred to as transdifferentiation) would circumvent difficulties associated with nuclear transplantation and be beneficial for producing replacement cells for therapeutic applications.

Reports on (partial) transdifferentiation of adult stem cells or somatic cells have broadened our view on the plasticity, or lack thereof, of committed or differentiated cells. In this communication, I briefly review the opinions on the transdifferentiation potential of adult stem cells. Examples of somatic cell transdifferentiation and epigenetic reprogramming are addressed. I also present a novel *in vitro* somatic cell reprogramming strategy recently developed in my laboratory and discuss the implications of observations there.

2. TRANSDIFFERENTIATION POTENTIAL OF ADULT STEM CELLS

Tissue-specific stem cells reside in adult tissues including blood, skin, central nervous system, liver, gastro-intes-

tinal tract or skeletal muscle (Blau *et al.* 2001). Adult stem cells are responsible for regenerating damaged tissue and maintaining tissue homeostasis, such as replenishment of skin and blood cells. In contrast to the pluripotent ES cells, the differentiation and regenerative potential of adult stem cells has traditionally been thought to be restricted to the tissues in which they reside.

However, it transpires that adult stem cells have a surprisingly broad differentiation ability (Blau *et al.* 2001). For example, bone marrow-derived cells not only replenish blood but also contribute to heart, brain, liver, muscle, skin and vascular endothelium. Other reports claim stem cell movement in the other direction with, for example, central nervous system-derived cells giving rise to blood. Similarly, neural stem cells can turn into skeletal muscle and hepatic stem cells into pancreas. Thus, it appears that in some situations, adult stem cells exhibit sufficient plasticity to differentiate into cell types outside their predicted developmental lineage, and can even cross germ layer boundaries (Anderson *et al.* 2001).

Despite the excitement resulting from the transdifferentiation potential of adult stem cells, some controversy remains regarding the plasticity of these cells. One issue is to demonstrate that transdifferentiated cells are actually derived from the stem cells of the donor tissue and not from contaminating cells. Notably, stem cell populations of haemopoietic origin have been identified in muscle (McKinney-Freeman *et al.* 2002). Unequivocal identification of a few stem cells in a large population of heterogeneous cells may be challenging and requires several markers. A second issue is to show that the transdifferentiated stem cells have normal chromosome contents. Co-culture of somatic cells or adult stem cells with ES cells leads to occasional cell fusion events that result in hyperdiploid hybrids which display an ES cell-like phenotype

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(Terada *et al.* 2002; Ying *et al.* 2002). The reversion of somatic nuclei to pluripotency following somatic cell fusion with ES cells has been documented (Tada *et al.* 2001). Cell fusion may therefore provide an explanation for how bone marrow cells or progenitor cells of the central nervous system can give rise to non-bone marrow or non-neural derivatives.

3. TRANSDIFFERENTIATION OF SOMATIC CELLS: CLASSICAL EXAMPLES

(a) *Transdifferentiation by cell transplantation*

It is well documented that somatic cells can alter their phenotype and function when placed into a new environment. In *Drosophila*, imaginal discs—non-stem cell larval structures determined to form specific adult body parts—can change their fate after regenerative cell divisions, a phenomenon specifically known as transdetermination (Wei *et al.* 2000). Transdetermination of disc cells also occurs after transplantation into a new tissue, such that leg cells convert to antenna cells, antenna to wing, etc. Transdetermination is believed to involve a misregulation of key genes, including *Hox* genes (Schneuwly *et al.* 1987). In amphibians, cells from the cornea can form a new lens after the original lens is removed during the tadpole stages (Schaefer *et al.* 1999; Henry & Elkins 2001). Transdifferentiation in this situation is controlled by factors secreted by the neural retina. This type of lens regeneration also occurs in the chick (Eguchi & Kodama 1993). Transdifferentiation can also take place after transplantation into ectopic sites in mammals. The injection of endothelial cells into damaged heart tissue causes their transdifferentiation into beating cardiomyocytes (Condorelli *et al.* 2001). Pancreatic epithelial progenitor cells can also differentiate into hepatocytes (Dabeva *et al.* 1997). It seems, therefore, that the microenvironment, including contact with surrounding cells, the extracellular matrix, the local milieu as well as growth and differentiation factors, plays a key role in redirecting cell fate. These factors may also influence stem cell fate as these cells migrate and enter other organs.

(b) *Parallels between mammalian stem cell fate change and transdetermination*

Although transdetermination in *Drosophila* does not involve stem cells, several parallels exist between mammalian stem cell fate change and disc cell transdetermination. First, both processes take place under regenerative proliferation. Second, progenitor cells, which can differentiate into mature effector cells, are involved. Third, cell fate changes in both situations do not lead to intermediate cell types but follow a normal pattern of differentiation. Last, *Hox* genes, implicated in transdetermination, are also preferentially expressed in adult stem cells and regulate stem cell behaviour (Sauvageau *et al.* 1995).

(c) *Transdifferentiation of somatic cells in culture*

Transdifferentiation of mammalian somatic cells has been achieved in co-culture or by manipulation of cell culture conditions. Myoblasts transdifferentiate into mature adipocytes by ectopic expression of adipogenic transcription factors under conditions permissive for adipogenesis (Hu *et al.* 1995). Induction of a hepatic transcription fac-

tor in pancreatic cells also causes their conversion into hepatocytes (Shen *et al.* 2000). Furthermore, inhibition of gap-junctional communication between cultured osteoblasts leads to an adipocytic phenotype (Schiller *et al.* 2001). Additionally, embryonic or neonatal umbilical vein endothelial cells transdifferentiate into beating cardiomyocytes when co-cultured with neonatal rat cardiomyocytes (Condorelli *et al.* 2001). Interestingly, endothelial cells derived from adults do not transdifferentiate in this system, suggesting that this kind of plasticity is age dependent.

(d) *Epigenetic reprogramming by cell hybridization*

During nuclear reprogramming, epigenetic modifications of the somatic nucleus must occur for pluripotency to be achieved. This presumably occurs after transplantation of a somatic nucleus into an oocyte. A differentiated nucleus can also be reprogrammed to a pluripotent state by fusion of a somatic cell with a stem cell. When mouse thymic lymphocytes are fused to EG cells, methylation of imprinted or non-imprinted genes and repetitive sequences is erased and the lymphocyte nuclei acquire pluripotential stem cell-like properties (Tada *et al.* 1997). Reversion of lymphocyte nuclei also occurs after fusion with ES cells; however, the methylation pattern of imprinted genes is maintained (Tada *et al.* 2001). Nevertheless, such fusions result in reactivation of a silent transgene (the ES cell-specific gene *Oct-4*) in the lymphocyte nucleus. Thus, factors in ES or EG cells can mediate some stage of epigenetic reprogramming of the somatic genome.

4. A NEW STRATEGY FOR TRANSDIFFERENTIATION

(a) *A reprogramming cell-free extract*

Extracts derived from gametes or somatic cells have been widely used to investigate various nuclear processes such as chromosome and nuclear envelope dynamics (Poccia & Collas 1996; Martins *et al.* 2000; Steen *et al.* 2000a,b) and chromatin remodelling (Kikyo *et al.* 2000). As nuclear structure can be extensively manipulated *in vitro*, we have developed cell extracts that proved to be useful for manipulating gene expression in nuclei or cells (Håkelién *et al.* 2002; Landsverk *et al.* 2002). Our system consists in incubating purified intact somatic nuclei or permeabilized cells in a nuclear and cytoplasmic extract from a different somatic cell type. Reprogramming extracts are prepared by cell sonication and sedimentation of the coarse material (Håkelién *et al.* 2002; Landsverk *et al.* 2002). The supernatant, or extract, provides nuclear regulatory components that mediate alterations in the gene expression profile of the target genome, while cytoplasmic components are, presumably, used to promote nuclear import of the nuclear regulatory components. The extract is supplemented with an ATP-regenerating system (ATP, creatine kinase and phosphocreatine) and GTP to promote physiological import of nuclear factors into the exogenous nuclei (see below).

(b) *Transcriptional activity in purified nuclei incubated in a somatic cell extract*

To demonstrate the viability of our *in vitro* system, we showed, using a non-radioactive nuclear run-on assay

Figure 1. Import of transcriptional activators of the *IL2* gene in 293T nuclei exposed to T-cell extract. (a) Nuclei from 293T cells (0 min) were incubated in stimulated T-cell extract and NFAT uptake was examined by immunofluorescence. Scale bar, 10 μ m. (b) Nuclear uptake of NFAT, AP-1, NF κ B, Oct-1 and BSA-NLS was examined by immunoblotting analysis of input 293T nuclei (input nuc.) and 293T nuclei exposed for 30 min to STE, UTE or in STE containing an antibody against nucleoporins (mAb414). Anti-histone H4 antibodies were used as loading control. Relative molecular masses of the bands are shown on the left.

(Patrone *et al.* 2000), that purified nuclei incubated in a somatic cell extract were capable of transcription. Nuclei purified by Dounce homogenization from 293T fibroblasts were incubated in a nuclear and cytoplasmic extract from 293T cells. The extract contained an ATP-regenerating system and each nucleotide in the form of NTPs. As the extract contains mRNA (Håkelién *et al.* 2002; P. Collas, unpublished data), biotin-labelled UTP was used as a marker of newly synthesized RNA. As a control, unlabelled UTP was used. After a 2 h incubation, biotinylated nascent RNAs were captured using Streptavidin beads. The RNA was eluted and used as a template for RT-PCR using β -actin transcript-specific primers. We showed that synthesis of β -actin mRNA takes place in the 293T nuclei. As millions of nuclei can be treated in a single experiment, purified nuclei may prove useful for (large-scale) transcription regulation studies *in vitro*, for investigating transcription factor binding to, for example, disease genes or for studying the molecular mechanisms of nuclear reprogramming.

(c) Nuclear uptake of transcription factors

As a first marker of nuclear reprogramming, we showed that the extract supports import of transcription factors into exogenous 293T nuclei. Nuclear import of transcription factors of the *interleukin-2* (*IL2*) gene (including NFAT and NF κ B) in STE and assembly of the AP-1 transcription complex occurred efficiently (Håkelién *et al.* 2002; Landsverk *et al.* 2002; figure 1). Notably, import of NFAT, NF κ B or assembly of AP-1 did not take place in UTE (figure 1b), despite import of a BSA-NLS substrate, indicating the specificity of import for the stimulated T-cell extract. Nuclear uptake was inhibited with an antibody against nucleoporins (figure 1b, mAb414), illustrating the physiological relevance of this nuclear import in the extract.

(d) Chromatin remodelling activity and induction of gene expression

As a second marker of reprogramming, we investigated the intranuclear anchoring and activity of the nucleosome remodelling complex, BAF (Zhao *et al.* 1998). A nuclear retention assay indicated that the BAF complex, initially present in a soluble (unbound) form in 293T cell nuclei,

rapidly associated with detergent-insoluble intranuclear structures (presumably chromatin and/or a 'matrix' network) upon incubation of the nuclei in the stimulated T-cell extract. Moreover, the stimulated T-cell extract elicited a β -actin-dependent ATPase activity of the BAF complex (Håkelién *et al.* 2002), suggestive of energy-dependent chromatin remodelling activities. It remains to be shown, however, whether the BAF complex actually alters nucleosome conformation in a manner similar to that of stimulated T cells in fibroblasts exposed to the extract.

The next marker of reprogramming examined was changes in chromatin composition. The potential for gene activity is generally associated with hyperacetylation of histone H4 in promoter regions (O'Neill & Turner 1995). Chromatin immunoprecipitation using antibodies against all forms of acH4 and dot-blot analysis using an oligonucleotide probe specific for the *IL2* promoter indicated that the extract promoted histone H4 acetylation at the *IL2* promoter in the fibroblast genome (figure 2a(i)). Again, H4 acetylation occurred specifically in extract from stimulated T cells (figure 2a, STE). Reprobing the dot-blot with a probe for the β -actin gene showed that the locus was H4-acetylated in all types of nuclei examined (figure 2a(ii)), consistent with the β -actin gene's being constitutively expressed.

Consistent with histone H4 acetylation at the *IL2* promoter, RT-PCR analysis indicated that transcription of the *IL2* gene was elicited in the 293T nuclei exposed to the stimulated extract (figure 2b). *IL2* transcription was also induced in nuclei from NT2 neuronal precursors, HeLa cells (not shown) and from HUVEC (figure 2b) exposed to the extract (Håkelién *et al.* 2002). This demonstrates that our nuclear reprogramming strategy is potentially applicable to target genomes of various cell types, both transformed and primary.

(e) Reprogramming fibroblast function in a somatic cell extract

Although purified nuclei may be useful for investigating mechanisms of reprogramming, their use is limited for therapeutic applications of *in vitro* reprogramming. Thus, we developed a procedure to reprogramme whole cells as opposed to purified nuclei. Our cell reprogramming

Figure 2. Alteration of chromatin structure in nuclei exposed to T-cell extract. (a) Histone H4 acetylation in the *IL2* promoter in 293T nuclei exposed to a Jurkat T-cell extract. Micrococcal nuclease-soluble chromatin was prepared from input 293T nuclei (input nuc.) and 293T nuclei incubated in UTE or STE. Acetylated H4 was immunoprecipitated and DNA isolated from anti-acH4 precipitate (bound) and supernatant (unbound) fractions. DNA was dot-blotted and hybridized to an *IL2* promoter-specific probe or a control β -actin probe. (b) Transcription of the *IL2* gene in 293T and HUVEC nuclei in T-cell extract. As controls, nuclei were incubated in extract containing mAb414 or 50 nM actinomycin D (Act.D). Nuclei were also exposed to extract treated with $100 \mu\text{g ml}^{-1}$ DNase I prior to transcription reaction. RNA was isolated from the reaction mix and *IL2* transcription examined by RT-PCR. (Reproduced, with permission, from Håkelién *et al.* (2002).)

Figure 3. *In vitro* cell reprogramming strategy. Healthy 'donor' cells (for example, fibroblasts) are reversibly permeabilized, reprogrammed in a test tube containing an extract of, for example, T cells, resealed and cultured.

approach is illustrated in figure 3. The plasma membrane of 'donor' cells (293T fibroblasts in our study) is reversibly permeabilized with the bacterial toxin Streptolysin O. Fibroblast function is reprogrammed by incubation of the permeabilized cells in a cytoplasmic and nuclear extract derived from, for example, T cells (or in principle any cell type) prepared as described above. T-cell-specific factors necessary to turn on T-cell-specific genes diffuse into the permeabilized fibroblasts and are actively taken up by the fibroblast nuclei. At the end of incubation, the cells are resealed with CaCl_2 and cultured. As illustrated below, analyses of cells incubated in the extract revealed the appearance of T-cell-like properties, which were stable for weeks following exposure to the extract.

(f) *Activation of a repressed gene*

Fibroblasts exposed to a T-cell extract change their gene expression pattern, as evidenced by cDNA macroarray analyses two weeks after reprogramming (Håkelién *et al.* 2002). Expression profile was compared with 293T cells permeabilized and exposed to a 293T extract. Expression of more than 120 genes was altered as a result of reprogramming. Haemopoietic genes, including interleukin and interleukin receptors, cytokines and cytokine receptors, chemokines and chemokine receptors, or

cell surface molecules, were activated or upregulated, whereas genes for fibroblast growth factors, adhesion molecules and cytoskeletal proteins were downregulated (Håkelién *et al.* 2002). Notably, levels of housekeeping gene mRNAs were not altered, indicating that changes in the transcription profile detected resulted specifically from reprogramming. Expression of the haemopoietic cell-specific marker genes *IL2*, *IL7*, *CD3*, *CD4* and *RANTES* and downregulation of the integrin $\beta 1$ gene (*ITGB1*) were verified by Northern blot analysis (figure 4).

(g) *Expression of cell surface antigens*

The reprogrammed 293T cells also express T-cell-specific surface receptors (Håkelién *et al.* 2002). These included CD3, CD4, CD45, the TCR and the β -subunit of IL-2R (*IL-2R β*), as shown by immunofluorescence (figure 5a). Morphological changes were also evident within the first few days of reprogramming, i.e. the fibroblasts appeared smaller and rounder and resembled T cells. Curiously, morphological changes were distinct for up to 7–10 days, after which the cells reverted to a fibroblast-like shape, although they continued to express T-cell-specific surface markers. The basis for this transient morphological alteration is being investigated.

Figure 4. 293T cells reprogrammed in a T-cell (Jurkat) extract express haemopoietic cell-specific genes. Northern blot analysis of IL-2, IL-7, CD3, CD4, RANTES, integrin β 1 and β -actin expression in 293T cells, Jurkat cells, 293T cells exposed to 293T extract and reprogrammed cells (three replicates, rep. 1–3). (Reproduced, with permission, from Håkelién *et al.* (2002).)

(h) Induction of a complex intracellular regulatory function

As a functional marker of reprogramming, we assessed the induction of a T-cell-specific biochemical pathway in the reprogrammed cells (Håkelién *et al.* 2002). Essentially, like T cells, all reprogrammed cells expressed the low-affinity IL-2R β (figure 5*b*(i,ii)). Upon stimulation of the TCR and CD3-signalling pathways in the reprogrammed cells (which normally occurs upon T-cell activation), we showed synthesis of the IL-2R α chain, which, presumably, contributed to the assembly of a functional high-affinity IL-2R (figure 5*b*(iii,iv)) (Håkelién *et al.* 2002). Thus, a complex T-cell-specific function was established in the reprogrammed cells. Whether other T-cell functions are also elicited upon reprogramming is under investigation. These observations predict that it may be possible to induce other complex (for example, secretory) functions in fibroblasts reprogrammed into other cell types. This functional aspect will be of crucial importance for therapeutic applications of *in vitro* cell reprogramming.

5. ARE MASTER-SWITCH GENES IMPORTANT FOR REPROGRAMMING?

Studies on transdifferentiation of somatic cells raises the suggestion that activation or suppression of so-called 'master genes' may be essential for the transition from one cell type to another. Long-term *in vivo* reconstitution of T-cell development in B cells by knocking out the *Pax5* gene has been reported, suggesting that *Pax5* is a master gene important for commitment to the B-cell lineage (Nutt *et al.* 1999; Rolink *et al.* 1999). *Pax5*^{-/-} pre-B cells express genes of different lineage-associated programmes, whereas restoration of *Pax5* activity represses lineage-promiscuous transcription. Similarly, the homeobox gene *PDX-1* has been proposed to be a master switch for initiating development of insulin-producing β -cells in the pan-

creas. *PDX-1* is essential for pancreas development and islet cell function (Jonsson *et al.* 1994). *In vivo* transfer of a *PDX-1* transgene into mouse liver was sufficient to convert a subpopulation of hepatocytes into insulin-producing β -like cells (Ferber *et al.* 2000), and suppression of *PDX-1* leads to a complete absence of the pancreas in *pdx-1* knockout mice. These observations suggest that *PDX-1* may be a master gene controlling β -cell function. However, it was recently reported that the transcription factor *Foxa2* acts upstream of *PDX-1* in the *PDX-1* regulatory cascade, and thereby controls expression of *PDX-1* (Lee *et al.* 2002). This argues that searching for master genes is no easy task. If such genes exist, their identification might allow for a more efficient transdifferentiation approach, provided one finds ways of manipulating their activity.

6. CONCLUSIONS AND PROSPECTS

(a) Innovative aspects of in vitro cell reprogramming

If *in vitro* cell reprogramming proves functional on a large scale, it would present innovative technological, commercial and societal benefits. (i) Current strategies for cell reprogramming or controlled differentiation (i.e. directed differentiation of stem cells) are based on a receptor-mediated approach. *In vitro* reprogramming uses permeabilized cells; thus 'reprogramming factors' access the cellular interior directly. This may not only render the strategy more effective, but has the advantage of being useful without having a great deal of prior knowledge of regulatory mechanisms controlling cell function. (ii) *In vitro* reprogramming may be applied to many cell types and thus has potential for treating many diseases. (iii) It may also allow for the production of isogenic (patient's own) cells for transplantation. (iv) Unlike reprogramming by nuclear transplantation into oocytes, somatic cells are used as a source of reprogramming material. Unlike eggs, these can be grown in large numbers and, if necessary, can be transformed to produce a consistent supply of reprogramming material. (v) Finally, ethical and legal issues regarding human cloning and production of ES cells from human embryos would be avoided.

Despite the promising results, it would be biased to say that everything is perfect. Our reprogramming assay involves extensive cell manipulation. Thus, application of this technology to produce replacement cells for therapeutic purposes requires significant developments and a large body of data is needed before this system can be applied to the generation of cells used for therapy in human patients.

(b) A 'therapeutic cell'?

It is clear from our observations that reprogramming of the 293T cells into T cells is not complete. For example, only a subset of genes is up- or downregulated and, to date, the reprogrammed cells do not express a pure T-cell-specific phenotype. Nevertheless, for cell replacement therapeutic applications, one may not need to produce a fully reprogrammed cell. For instance, for treating Type 1 diabetes, it may be sufficient to engineer a cell that secretes regulated levels of insulin in response to glucose stimulation, very much like a pancreatic β cell; however

Figure 5. 293T cells reprogrammed in T-cell (Jurkat) extract exhibit T-cell-specific markers and function. (a) 293T cells exposed to a control 293T extract or a Jurkat extract, and Jurkat cells were analysed 4 and 14 days after reprogramming by immunofluorescence using indicated FITC-conjugated antibodies. DNA was labelled with propidium iodide. (b) Induction of a T-cell-specific signalling pathway in reprogrammed cells. Jurkat cells, and 293T cells exposed to a 293T extract or Jurkat extract were stimulated with anti-CD3 antibodies and PMA. Stimulated and unstimulated cells were analysed by immunofluorescence using anti-IL2R β and anti-IL2R α antibodies. Scale bars, 10 μ m. (Adapted from Håkelién *et al.* (2002), with permission.).

the replacement cell may not need to be a β cell *per se*. This hypothesis, however, is purely speculative and will require extensive genetic and functional investigations. Nevertheless, we tentatively propose a concept that a partially reprogrammed cell that displays the necessary therapeutic properties, or *therapeutic cell*, may be what is required to treat certain diseases.

(c) Using in vitro reprogramming as a tool

Several laboratories have reported the transdifferentiation of stem cells or somatic cells into cells types beyond their normal, predicted, developmental lineage. Nonetheless, recent studies indicate that isolation of stem cells of cell fusion events may account for the high level of plasticity of adult stem cells. This underlines how little we know about genome plasticity and illustrates the limitations of reprogramming cell lineages without understanding the underlying molecular processes.

Our reprogramming assay may provide a powerful system for analysing nuclear reprogramming processes as they occur *in vitro*. It will be exciting to apply similar methods to the analysis of other systems such as ES or EG cells, and to the identification of molecules central to biological processes as diverse as the establishment of pluripotency or regulation of differentiation. A clearer

understanding of nuclear reprogramming will not only lead to a better appreciation of (de)differentiation, cloning or stem cell biology, but will also be relevant to the aberrant programming that occurs in cancer and other pathological situations.

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REFERENCES

- Anderson, D. J., Gage, F. H. & Weissman, I. L. 2001 Can stem cells cross lineage boundaries? *Nature Med.* **7**, 393–395.
- Blau, H. M., Brazelton, T. R. & Weimann, J. M. 2001 The evolving concept of a stem cell: entity or function? *Cell* **105**, 829–841.
- Condorelli, G. (and 18 others) 2001 Cardiomyocytes induce endothelial cells to transdifferentiate into cardiac muscle: implications for myocardium regeneration. *Proc. Natl Acad. Sci. USA* **98**, 10 733–10 738.
- Dabeva, M. D., Hwang, S. G., Vasa, S. R., Hurston, E., Novikoff, P. M., Hixson, D. C., Gupta, S. & Shafritz, D. A. 1997 Differentiation of pancreatic epithelial progenitor cells into hepatocytes following transplantation into rat liver. *Proc. Natl Acad. Sci. USA* **94**, 7356–7361.

- Eguchi, G. & Kodama, R. 1993 Transdifferentiation. *Curr. Opin. Cell Biol.* **5**, 1023–1028.
- Ferber, S. (and 10 others) 2000 Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. *Nature Med.* **6**, 568–572.
- Håkelién, A. M., Landsverk, H. B., Robl, J. M., Skålhegg, B. S. & Collas, P. 2002 Reprogramming fibroblasts to express T-cell functions using cell extracts. *Nature Biotechnol.* **20**, 460–466.
- Henry, J. J. & Elkins, M. B. 2001 Cornea-lens transdifferentiation in the anuran, *Xenopus tropicalis*. *Dev. Genes Evol.* **211**, 377–387.
- Hu, E., Tontonoz, P. & Spiegelman, B. M. 1995 Transdifferentiation of myoblasts by the adipogenic transcription factors PPAR gamma and C/EBP alpha. *Proc. Natl Acad. Sci. USA* **92**, 9856–9860.
- Jonsson, J., Carlsson, L., Edlund, T. & Edlund, H. 1994 Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* **371**, 606–609.
- Kikyo, N., Wade, P. A., Guschin, D., Ge, H. & Wolffe, A. P. 2000 Active remodeling of somatic nuclei in egg cytoplasm by the nucleosomal ATPase ISWI. *Science* **289**, 2360–2362.
- Landsverk, H. B., Håkelién, A. M., Küntziger, T., Robl, J. M., Skålhegg, B. S. & Collas, P. 2002 Reprogrammed gene expression in a somatic cell-free extract. *EMBO Rep.* **3**, 384–389.
- Lee, C. S., Sund, N. J., Vatamaniuk, M. Z., Matschinsky, F. M., Stoffers, D. A. & Kaestner, K. H. 2002 Foxa2 controls Pdx1 gene expression in pancreatic beta-cells *in vivo*. *Diabetes* **51**, 2546–2551.
- McKinney-Freeman, S. L., Jackson, K. A., Camargo, F. D., Ferrari, G., Mavilio, F. & Goodell, M. A. 2002 Muscle-derived hematopoietic stem cells are hematopoietic in origin. *Proc. Natl Acad. Sci. USA* **99**, 1341–1346.
- Martins, S. B., Eide, T., Steen, R. L., Jahnsen, T., Skålhegg, B. S. & Collas, P. 2000 HA95 is a protein of the chromatin and nuclear matrix regulating nuclear envelope dynamics. *J. Cell Sci.* **113**, 3703–3713.
- Nutt, S. L., Heavey, B., Rolink, A. G. & Busslinger, M. 1999 Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* **401**, 556–562.
- O'Neill, L. P. & Turner, B. M. 1995 Histone H4 acetylation distinguishes coding regions of the human genome from heterochromatin in a differentiation-dependent but transcription-independent manner. *EMBO J.* **14**, 3946–3957.
- Patrone, G., Puppo, F., Cusano, R., Scaranari, M., Ceccherini, I., Puliti, A. & Ravazzolo, R. 2000 Nuclear run-on assay using biotin labeling, magnetic bead capture and analysis by fluorescence-based RT-PCR. *Biotechniques* **29**, 1012–1017.
- Poccia, D. L. & Collas, P. 1996 Transforming sperm nuclei into male pronuclei *in vivo* and *in vitro*. *Curr. Topics Dev. Biol.* **34**, 25–88.
- Rolink, A. G., Nutt, S. L., Melchers, F. & Busslinger, M. 1999 Long-term *in vivo* reconstitution of T-cell development by Pax5-deficient B-cell progenitors. *Nature* **401**, 603–606.
- Sauvageau, G., Thorsteinsdottir, U., Eaves, C. J., Lawrence, H. J., Largman, C., Lansdorp, P. M. & Humphries, R. K. 1995 Overexpression of HOXB4 in hematopoietic cells causes the selective expansion of more primitive populations *in vitro* and *in vivo*. *Genes Dev.* **9**, 1753–1765.
- Schaefer, J. J., Oliver, G. & Henry, J. J. 1999 Conservation of gene expression during embryonic lens formation and cornea-lens transdifferentiation in *Xenopus laevis*. *Dev. Dyn.* **215**, 308–318.
- Schiller, P. C., D'Ippolito, G., Brambilla, R., Roos, B. A. & Howard, G. A. 2001 Inhibition of gap-junctional communication induces the trans-differentiation of osteoblasts to an adipocytic phenotype *in vitro*. *J. Biol. Chem.* **276**, 14 133–14 138.
- Schneuwly, S., Klemenz, R. & Gehring, W. J. 1987 Redesigning the body plan of *Drosophila* by ectopic expression of the homeotic gene Antennapedia. *Nature* **325**, 816–818.
- Shen, C. N., Slack, J. M. & Tosh, D. 2000 Molecular basis of transdifferentiation of pancreas to liver. *Nature Cell Biol.* **2**, 879–887.
- Steen, R. L., Cubizolles, F., Le Guellec, K. & Collas, P. 2000a A-kinase anchoring protein (AKAP)95 recruits human chromosome-associated protein (hCAP)-D2/Eg7 for chromosome condensation in mitotic extract. *J. Cell Biol.* **149**, 531–536.
- Steen, R. L., Martins, S. B., Tasken, K. & Collas, P. 2000b Recruitment of protein phosphatase 1 to the nuclear envelope by A-kinase anchoring protein AKAP149 is a prerequisite for nuclear lamina assembly. *J. Cell Biol.* **150**, 1251–1262.
- Tada, M., Tada, T., Lefebvre, L., Barton, S. C. & Surani, M. A. 1997 Embryonic germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells. *EMBO J.* **16**, 6510–6520.
- Tada, M., Takahama, Y., Abe, K., Nakastuji, N. & Tada, T. 2001 Nuclear reprogramming of somatic cells by *in vitro* hybridization with ES cells. *Curr. Biol.* **11**, 1553–1558.
- Terada, N., Hamazaki, T., Oka, M., Hoki, M., Mastalerz, D. M., Nakano, Y., Meyer, E. M., Morel, L., Petersen, B. E. & Scott, E. W. 2002 Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature* **416**, 542–545.
- Wei, G., Schubiger, G., Harder, F. & Muller, A. M. 2000 Stem cell plasticity in mammals and transdetermination in *Drosophila*: common themes? *Stem Cells* **18**, 409–414.
- Ying, Q. L., Nichols, J., Evans, E. P. & Smith, A. G. 2002 Changing potency by spontaneous fusion. *Nature* **416**, 545–548.
- Zhao, K., Wang, W., Rando, O. J., Xue, Y., Swiderek, K., Kuo, A. & Crabtree, G. R. 1998 Rapid and phosphoinositide-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. *Cell* **95**, 625–636.

GLOSSARY

- acH4: acetylated histone H4
 AP-1: activator protein 1
 BSA-NLS: bovine serum albumin—nuclear localization signal conjugate
 EG: embryonic germ
 ES: embryonic stem
 GTP: guanosine triphosphate
 HUVEC: human umbilical vein endothelial cells
 IL: interleukin
 IL-2R: interleukin-2 receptor
 NFAT: nuclear factor of activated T cells
 NFκB: nuclear factor κB
 NTP: nucleotide triphosphate
 PDX-1: pancreas duodenum homeobox-1
 RT-PCR: reverse transcriptase polymerase chain reaction
 STE: extract from stimulated T cells
 TCR: T-cell receptor
 UTE: extract from unstimulated T cells
 UTP: uridine triphosphate