PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Epidermal stem cells: markers, patterning and the control of stem cell fate

Fiona M. Watt

Phil. Trans. R. Soc. Lond. B 1998 **353**, 831-837
doi: 10.1098/rstb.1998.0247

The Royal Society

Epidermal stem cells: markers, patterning and the control of stem cell fate

Fiona M. Watt

Keratinocyte Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, UK

Within the epidermis, proliferation takes place in the basal layer of keratinocytes that are attached to an underlying basement membrane. Cells that leave the basal layer undergo terminal differentiation as they move towards the tissue surface. The basal layer contains two types of proliferative keratinocyte: stem cells, which have unlimited self-renewal capacity, and transit amplifying cells, those daughters of stem cells that are destined to withdraw from the cell cycle and terminally differentiate after a few rounds of division. Stem cells express higher levels of the β_1-integrin family of extracellular matrix receptors than transit amplifying cells and this can be used to isolate each subpopulation of keratinocyte and to determine its location within the epidermis. Variation in the levels of E-cadherin, β-catenin and plakoglobin within the basal layer suggests that stem cells may also differ from transit amplifying cells in intercellular adhesiveness. Stem cells have a patterned distribution within the epidermal basal layer and patterning is subject to autoregulation. Constitutive expression of the transcription factor c-Myc promotes terminal differentiation by driving keratinocytes from the stem cell compartment into the transit amplifying compartment.

Keywords: epidermis; stem cells; keratinocytes; integrins; cadherins; c-Myc

1. INTRODUCTION: WHAT IS AN EPIDERMAL STEM CELL?

During embryonic development, cell divisions give rise to new, differentiated cell types or increase the total number of cells in the embryo. In contrast, the major role of cell division in adult life is to replace cells that have been lost through death or injury, thereby maintaining cell number at an approximately constant level (Hall & Watt 1989). In some adult tissues the terminally differentiated cells have a short life span and are unable to divide: in such tissues cell replacement occurs through proliferation of a distinct subpopulation of cells, known as stem cells. A stem cell can be defined as any cell that retains a high capacity for self-renewal throughout adult life; in addition, stem cells usually have the ability to produce daughter cells that undergo terminal differentiation (Lajtha 1979).

Within mammalian epidermis there are two well-characterized terminal differentiation pathways, the endpoints of which are the cells of the hair shaft and the outermost, cornified layers of interfollicular epidermis. Under normal, steady-state conditions there are distinct stem cell populations maintaining each lineage. However, when the epidermis is injured, hair follicle keratinocytes can migrate over the denuded interfollicular basement membrane to repopulate the interfollicular epidermis (Al-Bawari & Potten 1976). Conversely, when interfollicular keratinocytes are grafted into an empty hair follicle in contact with dermal papilla cells they are able to form a hair shaft (Reynolds & Jahoda 1992). Thus, epidermal stem cells are certainly bipotential and they may also contribute to other, less well-characterized, epidermal differentiation compartments, such as the sweat gland (Miller *et al*. 1998).

There is a considerable amount of evidence, dating back over many years, that the proliferative compartment of epidermal keratinocytes is heterogeneous. Thus, when mouse epidermis is regenerated after ionizing or ultraviolet irradiation of the skin, only about 10% of basal keratinocytes are able to form detectable foci of new epidermis (Withers 1967; Potten & Hendry 1973), even though 60% of basal keratinocytes in mouse and human epidermis are cycling (Withers 1967; Potten & Hendry 1973; Potten & Morris 1988). The dividing cells with the ability to regenerate the epidermis are believed to correspond to stem cells and the others have been named transit amplifying cells (Potten 1974, 1981). Transit amplifying cells are defined as proliferating keratinocytes that have limited self-renewal capacity (corresponding to about three rounds of division) and a high probability of withdrawing from the cell cycle and undergoing terminal differentiation.

Further evidence for proliferative heterogeneity of epidermal keratinocytes has come from studies of cultured human keratinocytes. Autografts of cultured keratinocytes form histologically normal epidermis when used in the treatment of burns victims and the progeny of cells that had been in culture persist on the patient for many years after grafting, even when the source of the cells was non-hair-bearing skin (Gallico *et al*. 1984; Compton *et al*. 1989). The observations made with the grafts establish that stem cells can survive in culture and, furthermore, that cells from hair follicles are not essential for long-term maintenance of interfollicular epidermis.

When cultured keratinocytes are seeded at clonal density some cells found large, actively growing colonies of more than 2×10^4 cells within 2 weeks, whereas others form smaller colonies in which all or most of the cells

undergo terminal differentiation within that period (Barrandon & Green 1987; Green & Barrandon 1988; Jones & Watt 1993). Serial cultivation experiments (Barrandon & Green 1987) and grafting of cultured keratinocytes onto nude mice (Jones & Watt 1993) have established that the cells that form the large colonies are able to self-renew and to generate daughters that complete the terminal differentiation programme, leading to the conclusion that these clonogenic cells are stem cells. In contrast, the cells that undergo terminal differentiation after a few rounds of division have the expected properties of transit amplifying cells (Jones & Watt 1993).

In addition to the direct demonstrations of proliferative heterogeneity provided by irradiated mouse skin and cell culture experiments, indirect evidence comes from the study of label-retaining cells. Under steady-state conditions *in vivo*, epidermal stem cells are believed to divide infrequently and to have a long cell cycle time. Hence, epidermal stem cells in S-phase at the time when a mouse is injected with ^{3}H-thymidine might retain the radioactive label over many weeks, in contrast to more rapidly dividing cells (for discussion, see Potten & Morris (1988) and Cotsarelis *et al.* (1989, 1990)). The label-retaining cells have a non-random distribution in the epidermis, being concentrated in a region of the hair follicle known as the bulge (Cotsarelis *et al.* 1990) or in the centre of columns of keratinocytes in interfollicular epidermis. Although retention of ^{3}H-thymidine is often cited as a marker of epidermal stem cells, there are good reasons to be cautious about this interpretation. For example, label retention has rarely been correlated directly with proliferative potential (see Morris & Potten 1994) and it is possible that at least some of the labelled cells have arrested in S-phase as a consequence of radiation-induced DNA damage.

Based on these and other observations a working model for self-renewal in human interfollicular epidermis is shown in figure 1. All the dividing cells are shown as lying in the basal layer; although this is true for cultured sheets of human keratinocytes grown on tissue culture plastic (Dover & Potten 1983; Jones & Watt 1993), it is undoubtedly an over-simplification in the case of the epidermis, as some S-phase and mitotic keratinocytes are also found in the first suprabasal layers (see, for example, Van Neste *et al.* (1983), Lavker & Sun (1982) and Penneys *et al.* (1970)).

2. INTEGRINS AS STEM CELL MARKERS

Although there is substantial evidence for proliferative heterogeneity of keratinocytes, analysis of stem cell properties has been hampered by the lack of markers to distinguish stem cells from transit amplifying cells. The only molecular marker available until recently was keratin 19, which is expressed in that region of the hair outer root sheath where the stem cells are reported to lie (Lane *et al.* 1991). The ideal stem cell marker would be one that could be used not only to determine the location of stem cells within the epidermis, but could also be used to purify viable stem cells.

In a search for potential markers of human epidermal stem cells, we began by investigating extracellular matrix receptors of the integrin family. Keratinocytes express

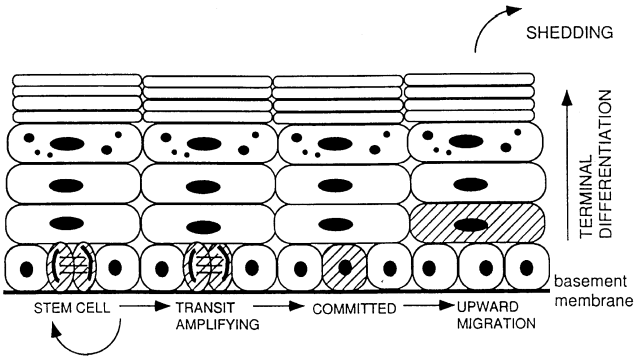

Figure 1. Diagram illustrating the organization of keratinocytes within human interfollicular epidermis (redrawn with permission from Jones & Watt (1993)). Stem cell divisions give rise both to stem cells and to transit amplifying cells. Daughters of transit amplifying cells withdraw from the cell cycle and undergo terminal differentiation as they move upwards to the surface of the epidermis.

several integrins, including $\alpha_2\beta_1$ (collagen receptor), $\alpha_3\beta_1$ (laminin receptor), $\alpha_5\beta_1$ (fibronectin receptor), $\alpha_6\beta_4$ (laminin receptor) and $\alpha_v\beta_5$ (vitronectin receptor) (reviewed by Watt & Hertle (1994)). Integrins not only mediate adhesion to the underlying extracellular matrix, but they also regulate the initiation of terminal differentiation (Adams & Watt 1989; Watt *et al.* 1993). Downregulation of integrin function and expression ensures that committed keratinocytes are selectively expelled from the basal layer and, in normal epidermis, integrin expression is confined to the basal layer (Adams & Watt 1990; Hotchin *et al.* 1995; reviewed by Watt & Hertle (1994)). The fact that integrins play a role in controlling epidermal differentiation and morphogenesis suggested that differences in integrin function or expression could provide markers for different subpopulations of proliferating basal cells.

The first way in which we tested the hypothesis that integrins might be stem cell markers was to use a fluorescence-activated cell sorter (FACS) to fractionate basal keratinocytes into three groups on the basis of surface integrin levels. The 20% of cells with the highest levels of the $\alpha_2\beta_1$ integrin had a fourfold higher efficiency in forming large, actively growing colonies (CFE) than the 20% of cells with the lowest levels, while the cells with the modal level of $\alpha_2\beta_1$ had the same CFE as unfractionated keratinocytes (Jones & Watt 1993). The same fold enrichment was obtained with an antibody that recognizes all β_1 integrins or with a combination of antibodies specific for $\alpha_2\beta_1$, $\alpha_3\beta_1$ and $\alpha_5\beta_1$. In contrast, less than twofold enrichment was obtained between the basal cells with the highest and lowest levels of $\alpha_6\beta_4$. The differences in integrin fluorescence were two- to threefold between the β_1-integrin-bright and β_1-integrin-dull basal cells and did not reflect differences in cell size. The maximum CFE obtained by this method was about 45%.

When plated on extracellular matrix proteins, keratinocytes adhere progressively over a period of 3 h (Adams & Watt 1991; figure 2). We found that the cells that adhered most rapidly to type IV collagen or fibronectin were enriched for cells capable of forming large, actively growing clones, whereas this relationship did not hold for laminin-1, a poorly adhesive substrate for keratinocytes

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

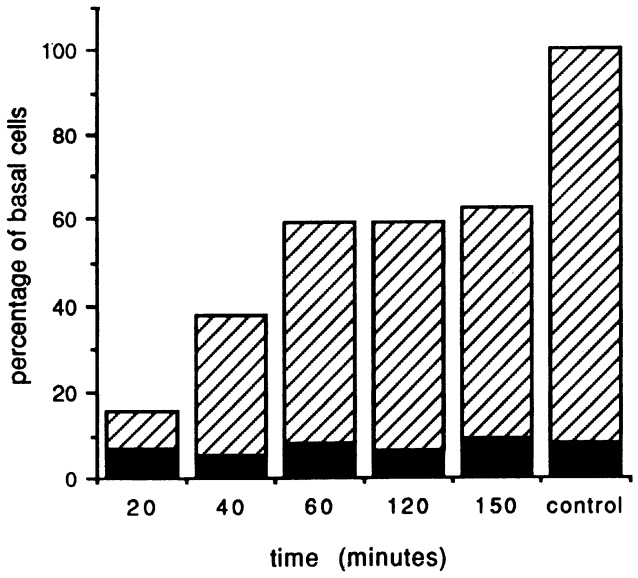

Figure 2. Adhesiveness and colony forming efficiency (reproduced with permission from Jones & Watt (1993)). Cultured human keratinocytes were plated into dishes coated with 100 μg ml^{-1} type IV collagen for the lengths of time shown, then harvested and replated to measure CFE. As a control, unfractionated keratinocytes were placed into the CFE assay without prior selection. The black portion of each bar represents cells that formed large, actively growing colonies. The percentage of total basal cells that attached at each time point is shown by the height of each bar. The hatched portion represents basal cells that attached but either did not divide or formed abortive colonies.

(Jones & Watt 1993; Karecla *et al.* 1994). The cells that adhered most rapidly had the highest level of β_1 integrins, consistent with the results of the FACS experiments. Using the adhesion-selection approach, CFEs of up to 91% could be obtained by adhesion for 20 min on 100 μg ml^{-1} type IV collagen.

We also examined the fate of the β_1-integrin-dull, slowly adhesive, basal keratinocytes. These cells tended to form small, abortive colonies of fewer than 32 cells in which the cells underwent terminal differentiation and expressed the differentiation marker involucrin within 14 days (Jones & Watt 1993). These cells thus have the predicted properties of transit amplifying cells. Figure 2 illustrates the relationship between adhesiveness and colony forming ability: with time of plating on type IV collagen the proportion of basal cells that attach increases; however, all of the cells that are clonogenic attach within 20 min.

Although the initial experiments were carried out with keratinocytes that had already been in culture for at least one passage, we obtained similar results with keratinocytes isolated directly from neonatal foreskin epidermis (Jones *et al.* 1995). Clonogenic keratinocytes could be isolated directly from skin on the basis of high surface levels of β_1 integrins and rapid adhesion to type IV collagen, whereas the basal cells with lower β_1 levels and slow adhesion to collagen were enriched for transit amplifying cells. The maximum CFE obtained for rapidly adhering cells isolated directly from skin was 63%.

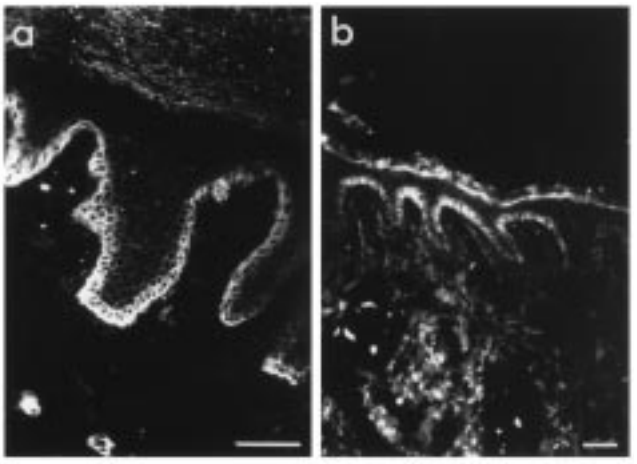

Figure 3. Variation in integrin levels in human skin from different body sites (reproduced with permission from Jones *et al.* (1995)). Frozen sections of (*a*) palm and (*b*) interfollicular scalp epidermis were labelled with fluorescence-conjugated antibodies to (*a*) $\alpha_3\beta_1$ or (*b*) $\alpha_2\beta_1$. Scale bars represent 50 μm.

3. STEM CELL PATTERNING

The two- to threefold differences in integrin levels on the surface of stem and transit amplifying cells can be visualized in sections of human epidermis by confocal immunofluorescence microscopy (Jones *et al.* 1995; Molès & Watt 1997). In the three body sites we have examined—neonatal foreskin, adult palm and interfollicular epidermis of adult scalp—patches (9–14 cells in diameter) of brightly fluorescent basal cells are interspersed with patches of basal cells of lower fluorescence. Patches can be visualized using antibodies to the β_1-integrin subunit or antibodies specific for $\alpha_2\beta_1$ or $\alpha_3\beta_1$.

The distribution of the integrin-bright patches is not random (figure 3). In foreskin and interfollicular scalp epidermis the bright patches are at the tips of the dermal papillae, where the dermis comes closest to the skin surface, whereas in palm, the bright patches are found at the tips of the deep rete ridges, where the epidermis projects furthermost into the skin. In scalp a 'cuff' of integrin-bright cells is also seen where the hair follicles open out onto the surface of the skin, and in the hair follicle the keratin-19-positive cells of the outer root sheath have higher $\alpha_2\beta_1$-integrin and $\alpha_3\beta_1$-integrin fluorescence than keratin-19-negative cells (Jones *et al.* 1995).

It is interesting that the integrin-bright cells lie in those regions of palm epidermis and hair follicle where stem cells have previously been located on the basis of ^{3}H thymidine labelling (Lavker & Sun 1982; Cotsarelis *et al.* 1989, 1990) or *in vitro* clonogenicity (Yang *et al.* 1993; Rochat *et al.* 1994). We used *in situ* hybridization with a cocktail of probes to histone mRNAs to determine the proportion of S-phase cells in different regions of the basal layer (Jones *et al.* 1995). In the palm, foreskin and interfollicular scalp epidermis the integrin-dull regions of the basal layer contain a significantly higher proportion of S-phase cells than the integrin-bright regions, consistent with the concept that, under steady-state conditions, stem cells divide infrequently (Potten & Morris 1988; Cotsarelis *et al.* 1990), despite being actively clonogenic *in vitro*.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The non-random distribution of stem cells within the basal layer of the epidermis could reflect a response of keratinocytes to differences in the local microenvironment or could be maintained by a keratinocyte-autonomous mechanism (see Hall & Watt (1989) for discussion). To distinguish between these possibilities we investigated whether or not patches of integrin-bright and integrin-dull cells formed in post confluent sheets of human keratinocytes cultured in the absence of dermis (Jones & Watt 1995). Labelling with antibodies to the α_2-integrin or α_3-integrin subunits revealed patches (about 11 cells in diameter) of integrin-bright cells. The size of the integrin-bright patches and the percentage of bright cells were similar whether integrin-bright or unfractionated keratinocytes were plated, and at densities ranging from 200 to 10^5 cells per 8 cm^2 dish. These results are shown schematically in figure 4 (in addition, see Jones (1997)).

The conclusion from these experiments is that there are patches of integrin-bright and integrin-dull cells within the basal layer of the epidermis and that their size and distribution can be determined by keratinocytes in the absence of signals from the underlying dermis or basement membrane. Nevertheless, it is clear that stem cells can respond to external cues. For example, both integrin-bright and integrin-dull keratinocytes will withdraw from the cell cycle and undergo terminal differentiation when deprived of extracellular matrix (Jones & Watt 1993). In addition, there is some evidence that the number of rounds of division that a transit amplifying cell undergoes prior to terminal differentiation may be influenced by plating density (Gandarillas & Watt 1997).

As discussed below, not all integrin-bright cells are stem cells and it is therefore possible that within an integrin-bright patch the stem cells are interspersed with transit amplifying cells. If, however, groups of stem cells do lie next to one another, an important implication is that the terminally differentiating cells in the suprabasal layers are not always the progeny of the cells directly beneath them. This would not be compatible with the 'epidermal proliferative unit' model of Potten (1974, 1981), in which a central stem cell surrounded by transit amplifying cells maintains a column of differentiating cells directly above it. Instead, a clustered distribution of stem cells would require some lateral migration of their progeny. In this context, it is interesting that clones of keratinocytes expressing mutant p53 in sun-exposed human epidermis are frequently conical, with the apex at the dermal–epidermal junction (Jonason *et al.* 1996).

4. ADDITIONAL MARKERS

Although integrins provide a valuable marker of epidermal stem cells, it is clear that not all integrin-bright basal keratinocytes are stem cells. In culture, the maximum CFE of rapidly adherent cells we have reported is 91% for cultured keratinocytes (Jones & Watt 1993) and 63% when cells are isolated directly from epidermis (Jones *et al.* 1995). The proportion of integrin-bright cells in the basal layer of the epidermis ranges from 25% in palm to $\geqslant$ 40% in foreskin and scalp (Jones *et al.* 1995), yet *in vivo* kinetic analysis and clonogenicity experiments with unfractionated keratinocytes would predict that the

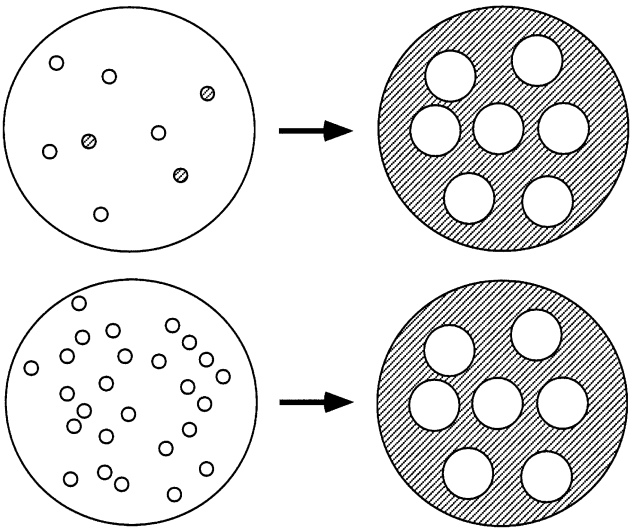

Figure 4. Diagram showing that the number and size of integrin-bright patches is independent of seeding density and starting population (stem or unfractionated). Integrin-bright cells (left) and integrin-bright patches (right) are shown as white; integrin-dull cells (left) and integrin-dull patches (right) are shown as hatched. Based on Jones (1997).

percentage of stem cells is closer to 10% (Potten & Morris 1988; Jones *et al.* 1995).

There are a variety of strategies that can be used to search for stem cell markers and one is to look for molecules whose expression varies between integrin-bright and -dull regions of the epidermal basal layer. Using this approach we have obtained some evidence that cell–cell adhesion molecules may provide additional stem cell markers (Molès & Watt 1997). The classical cadherins expressed by keratinocytes are E- and P-cadherin, which mediate calcium-dependent homophilic intercellular adhesion via adherens junctions (Hodivala & Watt (1994) and references cited herein). Previous experiments have shown that perturbation of cadherin function, by culturing keratinocytes in low calcium medium (Hodivala & Watt 1994) or introducing a dominant negative E-cadherin mutant (Zhu & Watt 1996), affects keratinocyte integrin expression. In addition, expression of the dominant-negative mutant results in a decrease in keratinocyte proliferation and increase in terminal differentiation, with evidence that these effects may be, at least in part, independent of the inhibition of cell–cell adhesion (Zhu & Watt 1996).

The cytoplasmic domains of E- and P-cadherin interact with a variety of proteins that regulate cadherin function, including α- and β-catenin and plakoglobin (γ-catenin) (reviewed by Aberle *et al.* (1996)). β-catenin and plakoglobin bind directly to the cadherin cytoplasmic domains and also bind α-catenin, which in turn is linked to the actin cytoskeleton. There is clear evidence that β-catenin and plakoglobin have signalling functions that may be independent of their role in regulating cell–cell adhesion (Gumbiner 1995; Barth *et al.* 1997).

When sections of palm and foreskin epidermis are stained with antibodies to E-cadherin, β-catenin or plakoglobin, patches of bright and dull cells are found; in contrast patchy staining is not observed with antibodies to P-cadherin or α-catenin or with antibodies to desmocollins

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

and desmogleins, cadherin components of desmosomal junctions (Molès & Watt 1997). The ratio of average fluorescence intensity between the bright and dull regions is approximately twofold. In both foreskin and palm the keratinocytes that are β_1-integrin-bright are E-cadherin-dull, β-catenin-dull and plakoglobin-bright.

Double labelling for β_1 integrins and β-catenin or plakoglobin establishes that there is approximate coincidence of the patches defined with integrin and catenin antibodies (Molès & Watt 1997), and thus the combination of E-cadherin and β_1-integrin antibodies would not be expected to give any greater enrichment for stem cells by FACS than β_1-integrin antibodies alone. However, the finding of heterogeneity in the levels of E-cadherin, β-catenin and plakoglobin is of interest because of the evidence outlined above that cadherins may regulate keratinocyte growth and differentiation and that there is potential cross-talk between cadherins and the β_1 integrins.

Our observations suggest that there may be gradients of cell–cell and cell–extracellular matrix adhesiveness within the basal layer of the epidermis (Jones & Watt 1993; Molès & Watt 1997). Cell adhesion may thus be an important component of what has been termed the stem cell 'niche' as put forward by Schofield (1978) and others (reviewed by Hall & Watt (1989)). The idea is that a stem cell resides in an optimal microenvironment, or niche. When a stem cell divides only one daughter cell can remain in the niche and the other will be committed to differentiate unless an additional niche is available. Components of the niche are believed to include neighbouring cells, extracellular matrix and diffusible factors such as growth factors (Hall & Watt 1989).

5. REGULATION OF STEM CELL FATE

Although we are clearly some way from being able to isolate a pure epidermal stem cell population, the discovery that integrin levels provide a marker for the stem cell compartment has already been useful in studies of molecules that potentially regulate stem cell fate. In recent experiments we have obtained evidence that c-Myc regulates exit from the stem cell compartment (Gandarillas & Watt 1997).

c-Myc is a member of the basic helix-loop-helix/leucine zipper family of DNA-binding proteins and regulates transcription through interactions with Max, another family member (reviewed by Amati & Land (1994)). Overexpression of c-Myc induces proliferation and inhibits differentiation in a variety of cell types (reviewed by DePinho *et al.* (1991)). In the epidermis and in culture, c-Myc is expressed by keratinocytes in the basal layer and is downregulated during terminal differentiation (Gandarillas & Watt 1995; Hurlin *et al.* 1995*a*,*b*) It has therefore been postulated that the function of c-Myc in the epidermis might be to promote proliferation and that c-Myc downregulation is required for the initiation of terminal differentiation.

To test whether constitutive expression of c-Myc might prevent exit from the stem cell compartment, we studied the consequences of infecting normal human epidermal keratinocytes with retroviral vectors expressing either wild-type c-Myc or a steroid-activatable construct, in which the ligand-binding domain of a mutant oestrogen receptor is fused to the carboxy terminus of c-Myc (MycER) (Littlewood *et al.* 1995). To our surprise we found that constitutive expression of c-Myc or activation of MycER with 4 hydroxytamoxifen (OHT) resulted in a reduction, rather than a stimulation, of keratinocyte proliferation (Gandarillas & Watt 1997). This was not due to induction of apoptosis, even though inappropriate expression of c-Myc is known to induce apoptosis in a variety of cell types (Harrington *et al.* 1994; Packham & Cleveland 1995). Instead, the proportion of terminally differentiated keratinocytes was increased.

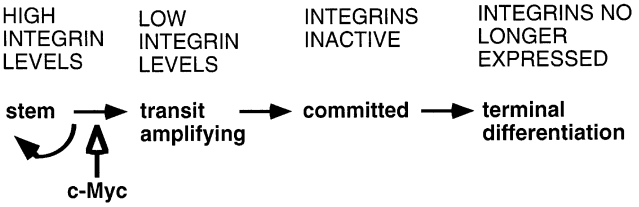

Figure 5. Flow diagram summarizing the role of c-Myc in regulating exit from the epidermal stem cell compartment.

When keratinocytes are stimulated to undergo terminal differentiation by placing them in suspension and depriving surface integrins of bound ligand, irreversible inhibition of proliferation occurs within 5 h and most cells express involucrin within 24 h (Adams & Watt 1989). In contrast, the kinetics of Myc-induced differentiation are quite different: Myc does not induce complete growth arrest and the proportion of differentiated cells does not rise markedly until 5 days after OHT induction of MycER. These observations are compatible with a role for c-Myc in driving exit from the stem to the transit amplifying compartment, because involucrin would be induced only as the transit amplifying divisions were completed over a period of a few days.

To test this hypothesis we examined the effects of Myc on the two available markers that distinguished stem cells and transit amplifying cells, namely surface levels of β_1 integrins and clonogenicity. When we compared untreated cells expressing MycER with those treated with OHT for 3 days we saw a twofold decrease in the surface level of total β_1 integrins (Gandarillas & Watt 1997), the same magnitude of decrease that occurs when cells move from the stem to the transit amplifying compartment (Jones & Watt 1993). OHT treatment of keratinocytes expressing MycER resulted in an increase in the proportion of cells that founded abortive clones, whether unfractionated or integrin-bright keratinocytes were used as the starting population. As a control in these experiments, keratinocytes were infected with a retrovirus encoding MycER with a deletion within the transcriptional regulatory domain: OHT treatment of these cells did not result in reduced integrin levels or in the formation of abortive colonies (Gandarillas & Watt 1997).

Our model for the role of c-Myc is shown schematically in figure 5. One prediction is that if cells are driven out of the stem cell compartment there should eventually be premature terminal differentiation and depletion of the proliferative population. This is indeed what we find when keratinocytes expressing MycER are allowed to reconstitute epidermis on a dermal equivalent: after 12

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

days of OHT treatment there is a decrease in the number of proliferating cells in the basal layer, clusters of involucrin-positive cells are found in the basal layer and there is also early onset of expression of the other differentiation markers keratin 10, loricin and filaggrin (Gandarillas & Watt 1997).

6. CONCLUSION AND PROSPECTS

Our recent findings provide, for the first time, a marker, namely the β_1 integrins, that can be used to isolate subpopulations of keratinocytes enriched for stem and transit amplifying cells. The distribution of the integrin-bright and integrin-dull populations within the epidermis points to a patterned distribution of stem and transit amplifying cells and this pattern can be established by keratinocytes in culture, in the absence of signals from the underlying dermis. c-Myc is the first factor that has been identified as regulating the transition from the stem to the transit amplifying compartment.

Goals for the future are to find additional stem cell markers so that the cells can be isolated to greater purity than at present, and to find additional molecules that regulate stem cell fate. Other important questions to be addressed are the extent to which high levels of integrin expression may be required for maintenance of the stem cell phenotype and identification of c-Myc target genes, including the tantalizing possibility that the target genes encode integrin subunits (Judware & Culp 1995, 1997).

It is a pleasure to acknowledge the major contributions of Philip Jones and Alberto Gandarillas to the data and ideas contained in this article. I thank Amrit K. Khalsa for typing the manuscript.

REFERENCES

Aberle, H., Schwartz, H. & Kemler, R. 1996 Cadherin–catenin complex: protein interactions and their implications for cadherin function. *J. Cell. Biochem.* **61**, 514–523.

Adams, J. C. & Watt, F. M. 1989 Fibronectin inhibits the terminal differentiation of human keratinocytes. *Nature* **340**, 307–309.

Adams, J. C. & Watt, F. M. 1990 Changes in keratinocyte adhesion during terminal differentiation: reduction in fibronectin binding precedes $\alpha_5\beta_1$ integrin loss from the cell surface. *Cell* **63**, 425–435.

Adams, J. C. & Watt, F. M. 1991 Expression of β_1, β_3, β_4 and β_5 integrins by human epidermal keratinocytes and non-differentiating keratinocytes. *J. Cell Biol.* **115**, 829–841.

Al-Bawari, S. E. & Potten, C. S. 1976 Regeneration and dose-response characteristics of irradiated mouse dorsal epidermal cells. *Int. J. Radiat. Biol.* **30**, 201–216.

Amati, B. & Land, H. 1994 Myc-Max-Mad: a transcription factor network controlling cell cycle progression, differentiation and death. *Curr. Opin. Genet. Dev.* **4**, 102–108.

Barrandon, Y. & Green, H. 1987 Three clonal types of keratinocyte with different capacities for multiplication. *Proc. Natn. Acad. Sci. USA* **84**, 2302–2306.

Barth, A. I. M., Näthke, I. S. & Nelson, W. J. 1997 Cadherins, catenins and APC protein: interplay between cytoskeletal complexes and signaling pathways. *Curr. Opin. Cell Biol.* **9**, 683–690.

Compton, C. C., Gill, J. M., Bradford, D. A., Regauer, S., Gallico, G. G. & O'Connor, N. E. 1989 Skin regenerated from cultured epithelial autografts on full-thickness burn wounds from 6 days to 5 years after grafting: a light, electron microscopic and immunohistochemical study. *Lab. Invest.* **60**, 600–612.

Cotsarelis, G., Cheng, S.-Z., Dong, G., Sun, T.-T. & Lavker, R. M. 1989 Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. *Cell* **57**, 201–209.

Cotsarelis, G., Sun, T.-T. & Lavker, R. M. 1990 Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell* **61**, 1329–1337.

DePinho, R. A., Schreiber-Agus, N. & Alt, F. W. 1991 *Myc* family oncogenes in the development of normal and neoplastic cells. *Adv. Cancer Res.* **57**, 1–46.

Dover, R. & Potten, C. S. 1983 Cell cycle kinetics of cultured human epidermal keratinocytes. *J. Invest. Dermatol.* **80**, 423–429.

Gallico, G. G. III, O'Connor, N. E., Compton, C. C., Kehinde, O. & Green, H. 1984 Permanent coverage of large skin wounds with autologous cultured epithelium. *N. Engl. J. Med.* **311**, 448–451.

Gandarillas, A. & Watt, F. M. 1995 Changes in expression of members of the *fos* and *jun* families and *myc* network during terminal differentiation of human keratinocytes. *Oncogene* **11**, 1403–1407.

Gandarillas, A. & Watt, F. M. 1997 c-Myc promotes differentiation of human epidermal stem cells. *Genes Devt* **11**, 2869–2882.

Green, H. & Barrandon, Y. 1988 Cultured epidermal cells and their use in the generation of epidermis. *News Physiol. Sci.* **3**, 53–56.

Gumbiner, B. M. 1995 Signal transduction by β-catenin. *Curr. Opin. Cell Biol.* **7**, 634–640.

Hall, P. A. & Watt, F. M. 1989 Stem cells: the generation and maintenance of cellular diversity. *Development* **106**, 619–633.

Harrington, E. A., Fanidi, A. & Evan, G. I. 1994 Oncogenes and cell death. *Curr. Opin. Genet. Dev.* **4**, 120–129.

Hodivala, K. J. & Watt, F. M. 1994 Evidence that cadherins play a role in the downregulation of integrin expression that occurs during keratinocyte terminal differentiation. *J. Cell Biol.* **124**, 589–600.

Hotchin, N. A., Gandarillas, A. & Watt, F. M. 1995 Regulation of cell surface β_1 integrin levels during keratinocyte terminal differentiation. *J. Cell Biol.* **128**, 1209–1219.

Hurlin, P. J., Foley, K. P., Ayer, D. E., Eisenman, R. N., Hanahan, D. & Arbeit, J. M. 1995*a* Regulation of Myc and Mad during epidermal differentiation and HPV-associated tumorigenesis. *Oncogene* **11**, 2487–2501.

Hurlin, P. J., Quéva, C., Koskinen, P. J., Steingrímsson, E., Ayer, D. E., Copeland, N. G., Jenkins, N. A. & Eisenman, R. N. 1995*b* Mad 3 and Mad 4: novel Max-interacting transcriptional repressors that surpress *c-Myc* dependent transformation and are expressed during neural and epidermal differentiation. *EMBO J.* **14**, 5646–5659.

Jonason, A. S., Kunala, S., Price, G. J., Restifo, R. J., Spinelli, H. M., Persing, J. A., Leffell, D. J., Tarone, R. E. & Brash, D. E. 1996 Frequent clones of p53-mutated keratinocytes in normal human skin. *Proc. Natn. Acad. Sci. USA* **93**, 14 025–14 029.

Jones, P. H. 1997 Epithelial stem cells. *BioEssays* **19**, 683–690.

Jones, P. H. & Watt, F. M. 1993 Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. *Cell* **73**, 713–724.

Jones, P. H., Harper, S. & Watt, F. M. 1995 Stem cell patterning and fate in human epidermis. *Cell* **80**, 83–93.

Judware, R. & Culp, L. A. 1995 Over-expression of transfected N-*myc* oncogene in human SKNSH neuroblastoma cells down-regulates expression of β_1 integrin subunit. *Oncogene* **11**, 2599–2607.

Judware, R. & Culp, L. A. 1997 Concomitant down-regulation of expression of integrin subunits by N-*myc* in human neuroblastoma cells: differential regulation of $\alpha 2$, $\alpha 3$ and β_1. *Oncogene* **14**, 1341–1350.

Karecla, P. I., Timpl, R. & Watt, F. M. 1994 Adhesion of human epidermal kertinocytes to laminin. *Cell Adhesion Commun.* **2**, 309–318.

Lajtha, L. G. 1979 Stem cell concepts. *Differentiation* **14**, 23–34.

Lane, E. B., Wilson, C. A., Hughes, B. R. & Leigh, I. M. 1991 Stem cells in hair follicles: cytoskeletal studies. *Ann. NY Acad. Sci.* **642**, 197–213.

Lavker, R. M. & Sun, T. T. 1982 Heterogeneity in epidermal basal keratinocytes: morphological and functional correlations. *Science* **215**, 1239–1241.

Littlewood, T. D., Hancock, D. C., Danielian, P. S., Parker, M. G. & Evan, G. I. 1995 A modified oestrogen receptor ligand-binding domain as an improved switch for the regulation of heterologous proteins. *Nucl. Acids Res.* **23**, 1686–1690.

Miller, S. J., Burke, E. M., Rader, M. D., Coulombe, P. A. & Lavker, R. M. 1998 Re-epithelialization of porcine skin by the sweat apparatus. *J. Invest. Dermatol.* **110**, 13–19.

Molès, J.-P. & Watt, F. M. 1997 The epidermal stem cell compartment: variation in expression levels of E-cadherin and catenins within the basal layer of human epidermis. *J. Histochem. Cytochem.* **45**, 867–874.

Morris, R. J. & Potten, C. S. 1994 Slowly cycling (label-retaining) epidermal cells behave like clonogenic stem cells *in vitro*. *Cell Proliferation* **27**, 279–289.

Penneys, N. S., Fulton, E. Jr, Weinstein, G. D. & Frost, P. 1970 Location of proliferating cells in human epidermis. *Arch. Derm.* **101**, 323–327.

Potten, C. S. 1974 The epidermal proliferative unit: the possible role of the central basal cell. *Cell Tissue Kinet.* **7**, 77–88.

Potten, C. S. 1981 Cell replacement in epidermis (keratopoiesis) via discrete units of proliferation. *Int. Rev. Cytol.* **69**, 271–318.

Potten, C. S. & Hendry, J. H. 1973 Clonogenic cells and stem cells in the epidermis. *Int. J. Radiat. Biol.* **24**, 537–540.

Potten, C. S. & Morris, R. J. 1988 Epithelial stem cells *in vivo*. *J. Cell Sci.* **10**(Suppl.), 45–62.

Reynolds, A. J. & Jahoda, C. 1992 Cultured dermal papilla cells induce follicle formation and hair growth by transdifferentiation of an adult epidermis. *Development* **115**, 587–593.

Rochat, A., Kobayashi, K. & Barrandon, Y. 1994 Location of stem cells of human hair follicles by clonal analysis. *Cell* **76**, 1063–1073.

Schofield, R. 1978 The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* **4**, 7–25.

Van Neste, D., Stagnet, M. J., Viac, J., Lachapelle, J. M. & Thivolet, J. 1983 A new way to evaluate the germinative compartment in human epidermis, using [^{3}H]thymidine incorporation and immunoperoxidase staining of 67K polypeptide. *Br. J. Dermatol.* **108**, 433–439.

Watt, F. M. & Hertle, M. D. 1994 Keratinocyte integrins. In *The keratinocyte handbook* (ed. I. M. Leigh, E. B. Lane & F. M. Watt), pp. 153–164. Cambridge University Press.

Watt, F. M., Kubler, M.-D., Hotchin, N. A., Nicholson, L. J. & Adams, J. C. 1993 Regulation of keratinocyte terminal differentiation by integrin–extracellular matrix interactions. *J. Cell Sci.* **106**, 175–182.

Withers, H. R. 1967 Recovery and repopulation *in vivo* by mouse skin epithelial cells during fractionated irradiation. *Radiat. Res.* **32**, 227–239.

Yang, J.-S., Lavker, R. M. & Sun, T.-T. 1993 Upper human hair follicle contains a subpopulation of keratinocytes with superior *in vitro* proliferative potential. *J. Invest. Dermatol.* **101**, 652–659.

Zhu, A. J. & Watt, F. M. 1996 Expression of a dominant negative cadherin mutant inhibits proliferation and stimulates terminal differentiation of human epidermal keratinocytes. *J. Cell Sci.* **109**, 3013–3023.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES