ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Developmental Biology 258 (2003) 141–153

DEVELOPMENTAL BIOLOGY

www.elsevier.com/locate/ydbio

Control of epidermal stem cell clusters by Notch-mediated lateral induction☆

Nicholas J. Savill[1,*] and Jonathan A. Sherratt

Centre for Theoretical Modelling in Medicine, Department of Mathematics, Heriot-Watt University, Edinburgh, EH14 4AS, UK

Received for publication 6 November 2002, revised 6 January 2003, accepted 30 January 2003

Abstract

Stem cells in the basal layer of human interfollicular epidermis form clusters that can be reconstituted in vitro. In order to supply the interfollicular epidermis with differentiated cells, the size of these clusters must be controlled. Evidence suggests that control is regulated via differentiation of stem cells on the periphery of the clusters. Moreover, there is growing evidence that this regulation is mediated by the Notch signalling pathway. In this paper, we develop theoretical arguments, in conjunction with computer simulations of a model of the basal layer, to show that regulation of differentiation is the most likely mechanism for cluster control. In addition, we show that stem cells must adhere more strongly to each other than they do to differentiated cells. Developing our model further we show that lateral-induction, mediated by the Notch signalling pathway, is a natural mechanism for cluster control. It can not only indicate to cells the size of the cluster they are in and their position within it, but it can also control the cluster size. This can only be achieved by postulating a secondary, cluster wide, differentiation signal, and cells with high Delta expression being deaf to this signal.

Keywords: Epidermis; Notch; Stem cells; Computer simulation

Introduction

Keratinocytes are the principal cell type of interfollicular epidermis. They are shed at the skin surface and replaced by division in the bottom-most layer of the epidermis, known as the basal layer. This layer undulates along the boundary between the epidermis and dermis.

There are three main types of keratinocytes in the basal layer of interfollicular epidermis: stem cells, transit-amplifying cells, and committed cells (Dover and Potten, 1983; Barrandon and Green, 1985, 1987). They can be partially sorted by their β1-integrin expression and the time it takes for them to adhere to type IV collagen (Jones and Watt, 1993; Jones et al., 1995; Gandarillas and Watt, 1997; Zhu and Watt, 1999). β1-integrin is a cell-membrane receptor that mediates adhesion to the extracellular matrix (ECM). Stem cells are integrin-bright and adhere rapidly to type IV collagen, whereas transit-amplifying cells are, on average, less bright and take longer to adhere, and committed cells are integrin-dull or negative. Using β1-integrin expression levels, Jones et al. (1995) and Jensen et al. (1999) have shown that stem cells reside in discrete clusters at the tips of dermal papillae of foreskin, breast, and scalp and at the tips of rete pegs of the palm. The number of stem cells in these clusters is unknown but probably numbers up to 40 (Jensen et al., 1999; Janes et al., 2002). In other work, Asplund et al. (2001) have shown that paternal and maternal X-chromosome inactivation has a mosaic-like pattern in normal human epidermis. The size of the mosaic tiles vary from 20 to 350 cells, suggesting that the size of human epidermal proliferative units (EPU; Potten, 1974, 1981) is at least of the order of 20 cells.

Stem cells divide infrequently either because they have long cell-cycle times or because some are arrested in G_0 phase. Given an appropriate signal, stem cells on

☆ Supplementary data associated with this article can be found at doi:10.1016/S0012-1606(03)00107-6.

* Corresponding author. Fax: +01223-336676.

E-mail address: njs@zoo.com.ac.uk (N.J. Savall).

[1] Present address: Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK.

0012-1606/03/$ – see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0012-1606(03)00107-6

the periphery of clusters differentiate into transit-amplifying cells (Lowell et al., 2000). This can occur at any time during the cell cycle (Dover and Watt, 1987). Transit-amplifying cells divide for another three to five generations. They divide at a faster rate than stem cells in vivo (Potten et al., 1982), though in culture, stem and transit-amplifying cells divide at the same rate (Dover and Potten, 1988). Postmitotic cells are committed to terminal differentiation (Adams and Watt, 1989, 1990; Hotchin et al., 1995). This involves migration from the basal layer (Jensen et al., 1999), the synthesis of keratin, involucrin, filligrin, and loricrin, and loss of the nucleus (Fuchs, 1990). After several weeks, they are shed at the skin surface.

The overall picture of interfollicular epidermis emerging from the work of Watt and coworkers is of islands of stem cells in a sea of differentiated cells (Jones et al., 1995; Jensen et al., 1999). Cells flow from the stem cell clusters through the transit-amplifying compartment (Jonason et al., 1996; Ren et al., 1997; Jensen et al., 1999) and up into the terminally differentiated layers. Although the stem cell clusters lie either at the tips of the dermal papillae or the rete pegs, these structures are not required for the formation and maintenance of the clusters (Jones et al., 1995).

A key ingredient of this epidermal structure is the mechanism that causes a stem cell to differentiate into a transit-amplifying cell. There is strong evidence that a differentiation signal comes from the Notch cell–cell signalling pathway (Lowell et al., 2000; Lowell and Watt, 2001; Rangarajan et al., 2001). Other factors implicated in stem cell fate are β1-integrin expression (Zhu et al., 1999; Levy et al., 2000; Brakebusch et al., 2000; Raghavan et al., 2000), c-Myc (Gandarillas and Watt, 1997; Arnold and Watt, 2001), and β-catenin (Zhu and Watt, 1999). The Notch receptor and its ligand, Delta, are *trans*-membrane proteins. Binding of Delta on one cell can activate Notch on a neighbouring cell. It is thought that the intracellular domain of the Notch receptor is cleaved, which then promotes transcription of, for example, Enhancer of split genes (for a review, see Baron et al., 2002). Notch signalling is implicated in many developmental processes (Lewis, 1996; Bray, 1998; Artavanis-Tsakonas et al., 1999; Baron et al., 2002).

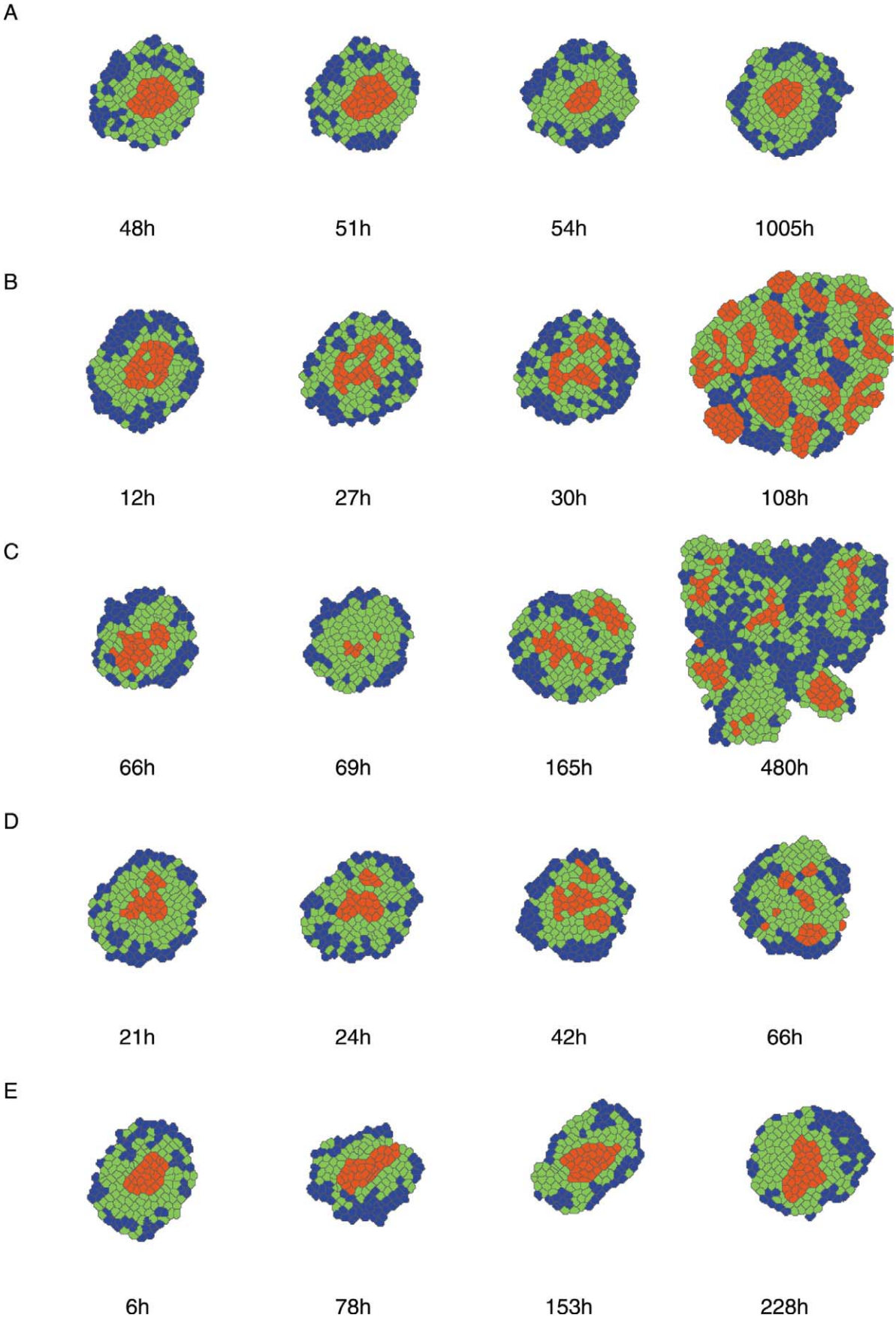
Lowell et al. (2000) found that human stem cells express about twice as much Delta mRNA as transit-amplifying cells, and committed cells express none. Notch mRNA was observed in all epidermal keratinocytes and was upregulated in terminally differentiated cells. By overexpressing Delta in keratinocytes, they discovered three major effects. First, a cell expressing high levels of Delta induces differentiation in its neighbouring cells. Second, a cell expressing high levels of Delta is deaf to the differentiation signal. And finally, stem cell clones overexpressing Delta form more compact clusters than wildtype clones, control clones, and transit-amplifying cell clones overexpressing Delta. Rangarajan et al. (2001) have found that mouse keratinocyte differentiation is induced by high Notch activation. As well as the Notch signalling pathway, many other molecular networks and signalling pathways are known to regulate epidermal growth and differentiation. These include NF-κB (Kaufman and Fuchs, 2000), wnt/β-catenin (Oro and Scott, 1998), Sonic hedgehog (Oro and Scott, 1998), 14-3-3σ (Dellambra et al., 2000), and α-catenin (Vasioukhin et al., 2001).

The basal layer of interfollicular epidermis is, therefore, a very dynamic environment. Cells divide, differentiate, migrate, jostle, squeeze, push, and stick to each other. This raises two related questions. What are the general rules that control cluster size and shape, and what is the mechanism that allows clusters to autonomously regulate their size? In Results, we study the first question using theoretical arguments and simulations of a model of cluster dynamics. The second question has a partial answer already, namely the Notch signalling pathway. We explore this idea in more detail later in Results.

To answer these questions, we develop a spatial model of stem cell clusters in the basal layer of interfollicular epidermis. Other modelling studies of the spatial structure of interfollicular epidermis include a model of the EPU of mice (Loeffler et al., 1987), a model of epidermal remodelling in psoriasis (Iizuka et al., 1996), and a topological model of division and migration of cells of the basal layer (Dubertret and Rivier, 1997).

We first consider some theoretical arguments for cluster size control.

Fig. 1. Control of cluster size. (A–C) Control mediated by regulation of differentiation. When the cluster size passes 30 cells, all cells on the periphery of the cluster immediately differentiate into transit-amplifying cells. (A) An example of a stable cluster. In the second frame (51 h), a cell division occurs taking the cluster size to 30 cells. All peripheral cells differentiate causing a reduction in the size (4 h). The simulation has been run for over 1000 h with no fragmentation or deformation of the cluster. (B) If interior cells differentiate when the cluster reaches a size of 30 cells (12 h), the cluster fragments (30 h), leading to uncontrolled growth (108 h). (C) If stem cells adhere equally to differentiated cells as they do to themselves, the cluster does not form a rounded shape (66 h). Individual cells can break off from the cluster, or, when differentiation occurs (69 h), the cluster fragments (165 h). This leads to uncontrolled growth (480 h). (D, E) Control mediated by regulation of division. Peripheral stem cells have a probability p per hour of differentiating, whatever the cluster size. When the cluster size is below 30 cells, all stem cells divide. When the cluster size is equal to or above 30 cells, all stem cells stop dividing. (D) In the first frame (21 h), differentiation causes the cluster to become misshapen ($p = 0.05$). A differentiation event (24 h) causes the cluster to fragment. This process continues (42 h), leading to uncontrolled growth (66 h). (E) By only allowing cells with less than 23 surface contacts with other stem cells to differentiate, a rounded shape is maintained and the cluster is stable. However, this model is not robust to changes in p or the number of surface contacts. Stem cells are red, transit-amplifying cells are green, and committed cells are blue.


A
48h
51h
54h
1005h
B
12h
27h
30h
108h
C
66h
69h
165h
480h
D
21h
24h
42h
66h
E
6h
78h
153h
228h

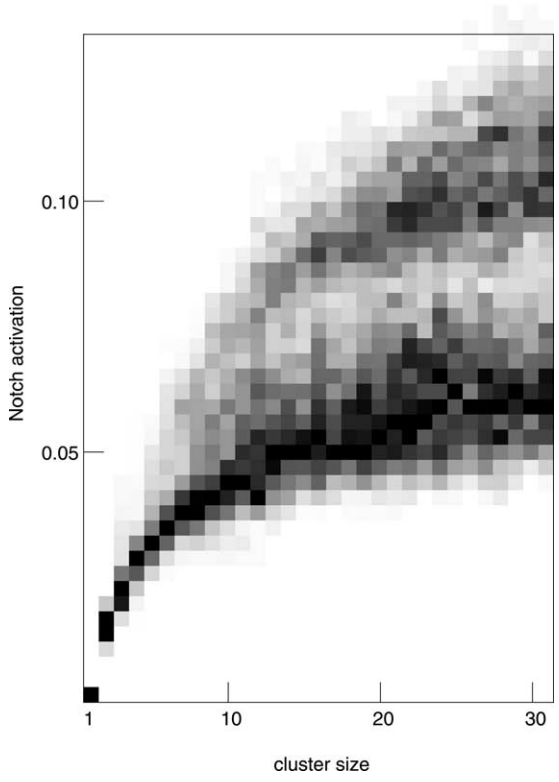


Fig. 2. The distribution of Notch activation (total bound receptor) in cells against the cluster size with lateral-induction. Small clusters, $\leqslant 5$ cells, only contain peripheral cells and their Notch activation is low. In large clusters with interior and peripheral cells, interior cells have a high Notch activation because they are completely surrounded by stem cells and peripheral cells have a lower Notch activation: hence the bimodal distribution at large cluster sizes. The graph is produced by making all cells in a cluster, except one, differentiate when the cluster size passes 30 cells. The simulation is run for 600 h. The frequency of cell Notch activation (bin width 0.003) for each cluster size is calculated. For each cluster size, the frequency of each bin is normalised, so that the modal bin has a frequency of one. Higher frequency corresponds to darker shading. Parameter values used are $\beta_1 = 0.001$, $\beta_2 = 1$, $\beta_3 = 1$, $\beta_4 = 1$, and $\beta_5 = 0.1$.

Materials and methods

Modelling active cluster size control

Our model of basal cell dynamics is based on the Graner and Glazier (1992) framework. This framework gives us the ability to model thousands of individual cells in space. Each cell has its own set of properties, like size, age, type, cell-cycle time, differentiation rate, adhesion strength to neighbouring cells, and Delta and Notch concentration on its membrane. We can also model cell division, growth, differentiation, migration, and loss.

The framework was developed to study differential adhesion and cell sorting in confluent sheets of cells (Graner and Glazier, 1992, 1993). It has been extended to study morphogenesis and taxis in *Dictyostelium discoideum* (Savill and Hogeweg, 1997; Marée et al., 1999), avascular tumour growth (Stott et al., 1999), and migrating fronts of cancer cells (Turner and Sherratt, 2002). Here, we further extend the model by adding localised Delta and Notch concentrations on the cell membranes.

Briefly, the cells are represented as extended objects on a square lattice. Each cell is assigned a unique number and type (e.g., stem). Adhesion between cell types is incorporated by defining surface energies between neighbouring cell membranes. Cells sort by minimising their surface energies under the constraint of maintaining a target area. Cell division is modelled by assigning half of the lattice points of a cell a new unique number. Growth is modelled by increasing the target area of a cell. Differentiation is modelled by assigning a cell a new type. Death or migration out of the basal layer is modelled by setting the target area of a cell to zero.

We assume that stem and transit-amplifying cell-cycle times are gamma distributed with a mean of 15.6 h and standard deviation of 3.7 h (taken from in vitro experiments of human keratinocytes; Dover and Potten, 1988). Transit-amplifying cells are limited to three divisions before ceasing to divide and becoming committed cells. The time a committed cell resides in the basal layer is exponentially distributed with a mean and standard deviation of 6.6 h (Dover and Potten, 1988).

There are some limitations to the Graner-Glazier model in general and our implementation of it. The model is a two-dimensional representation of a layer of cells. Therefore, there is no movement in the third dimension. Moreover, cells only form adhesive contacts on their lateral surfaces, so there is no representation of adhesive contacts on the basal or apical surfaces of the cells.

The model of Notch mediated lateral-induction

Delta-Notch cell–cell signalling is incorporated into the Graner-Glazier model by solving a system of simultaneous ordinary differential equations defined on the boundaries of stem cells with neighbouring stem cells.

The dedimensionalised equations of the model of lateral-induction are

$$\frac{d}{dt}x_{i,j} = \beta_1 + \beta_2\frac{V_\sigma}{S_\sigma} - \left(1 - \frac{Y_{i,j}}{s_{i,j}}\right)x_{i,j} + \beta_3\frac{Y_{i,j}}{S_{i,j}} - \beta_4 x_{i,j}, \quad (1)$$

$$\frac{d}{dt}y_{i,j} = (1 - y_{i,j})\frac{X_{i,j}}{s_{i,j}} - \beta_3 y_{i,j} - \beta_5 y_{i,j}, \quad (2)$$

where

$$X_{i,j} = \sum_{(k,l)\in\Gamma_{i,j}} x_{k,l}, \quad (3)$$

$$Y_{i,j} = \sum_{(k,l)\in\Gamma_{i,j}} y_{k,l}, \quad (4)$$

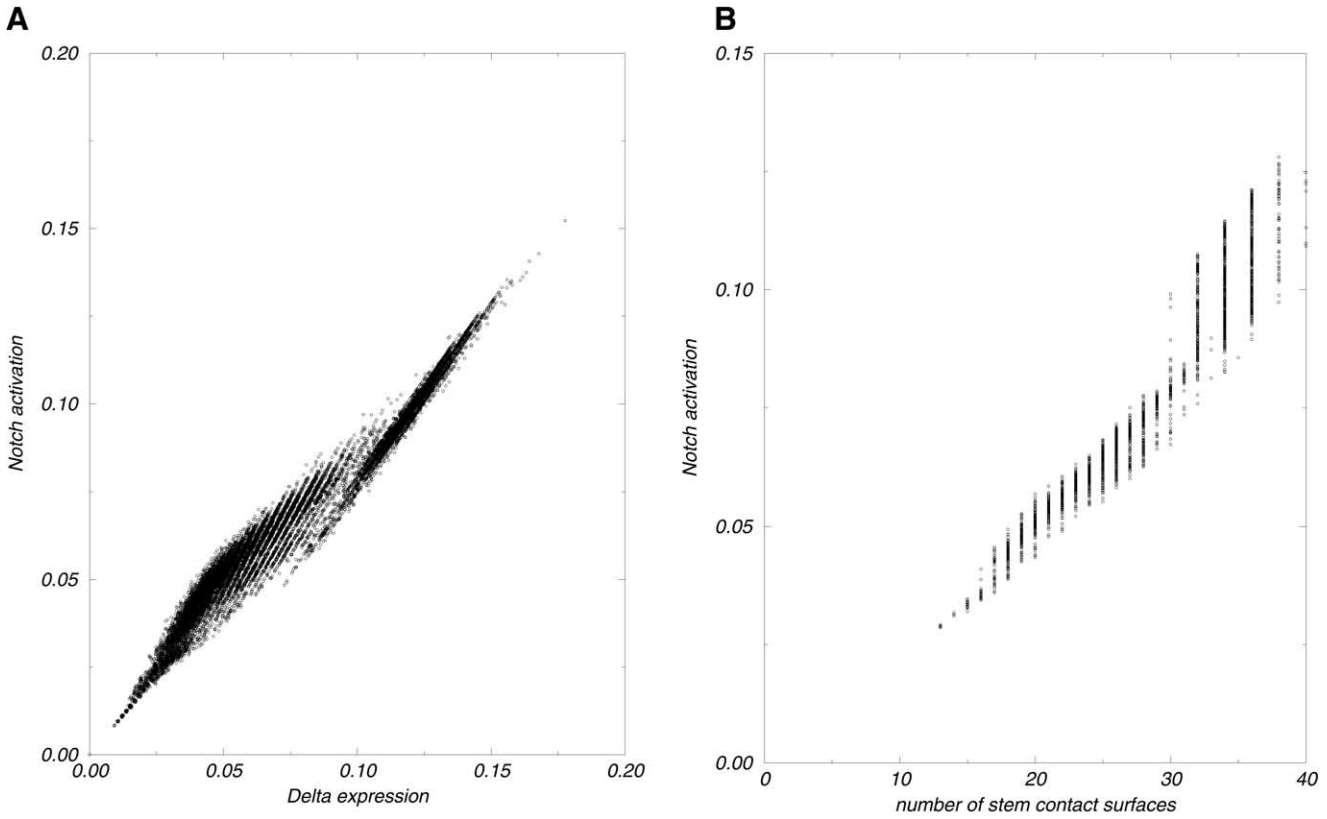


Fig. 3. (A) Notch activation is highly correlated which Delta expression in individual cells. (B) Notch activation of individual cells against the number of their surfaces in contact with other stem cells. Interior cells have the most number of surface contacts and hence, the largest activation, peripheral cells have fewer surface contacts and hence, lower activation. Data taken from simulations as in Fig. 1.

$$U_\sigma = \sum_{(k,l)\in\Omega_\sigma} s_{k,l}x_{k,l}, \tag{5}$$

and

$$V_\sigma = \sum_{(k,l)\in\Omega_\sigma} s_{k,l}y_{k,l}. \tag{6}$$

The variable $x_{i,j}$ is the dedimensionalised Delta expression on surfaces at position (i,j). The variable $y_{i,j}$ is the dedimensionalised bound Notch receptor (Delta-Notch complex) on surfaces at position (i,j).

For each lattice point (i,j) of a particular cell σ, the first term in Eq. (1) is the background production rate of Delta in the cell. The second term is the production rate of Delta induced by the level of Notch activation in the cell. The third term is the reaction rate of Delta in the cell with free Notch on neighbouring cells. The fourth term is the disassociation rate of the Delta-Notch complex, and the final term is the decay rate of Delta. The first term in Eq. 2 is the reaction rate of Delta on neighbouring cells with free Notch in the cell. The second term is the disassociation rate of the Delta-Notch complex. The final term is the internalisation rate of bound Notch.

We assume that receptor density is constant and uniform on a cell's membrane and that Delta expression in a cell is proportional to the Notch activation in that cell [hence the factor $1 - y$ in Eqs. (1) and (2)].

Ω_σ is the set of all lattice points on cell σ's membrane. A lattice point on the membrane of a cell may have multiple surfaces. $s_{i,j}$ is the number of surfaces associated with lattice point (i,j), $S_\sigma = \Sigma_{(i,j)\in\Omega_\sigma} s_{i,j}$ is the total number of surfaces of cell σ. $\Gamma_{i,j}$ is the set of lattice points neighbouring lattice point (i,j) that belong to stem cells that are different from the cell at point (i,j). U_σ is the dedimensionalised total Delta expression in cell σ. V_σ is the dedimensionalised total bound Notch in cell σ.

These equations are solved until they reach equilibrium using a second order Runge-Kutta method with a timestep of 0.5. Cell fate is based on the equilibrium values of U_σ and V_σ.

Results

Clusters actively control their size by regulating differentiation

Theoretical considerations

Our first question is, what general rules control cluster size and shape? A cluster that becomes too small will not

produce enough differentiated cells to supply the local epidermis. Conversely, a cluster that grows without bounds or clusters that continually fragment may cause abnormal tissue growth, as seen in diseases like psoriasis. Let us first consider cluster size control without concerning ourselves yet about the biochemical details.

The first possibility is that something in the local environment of dermal papillae or rete pegs externally regulates cluster size. However, apparently normal stratified epidermis can be reconstituted in vitro without these structures. Jones et al. (1995) have grown confluent sheets of keratinocytes on plastic. They found that patches of integrin bright cells from these sheets had the same variation in fluorescent intensity and diameter as patches sampled from skin. And the percentage of bright cells between the sheets and skin were very similar.

So external control of cluster size appears unlikely. This implies that clusters must control their own size via rates of division and differentiation and maybe, to a small extent, apoptosis (Laporte et al., 2000; Savill, 2003).

One possibility that can be discounted is that stem cell-cycle time is identical to the time it takes stem cells to differentiate. On the face of it this would keep the number of stem cells in a cluster constant. However, there are many problems with this idea. First, it is highly unlikely that two independent biological processes have the exact same rate. Second, keratinocytes are known to have large variances in their cell-cycle time (Duffill et al., 1976; Dover and Potten, 1988). And it is not unreasonable to think that there is also variance in differentiation times. This means that the mean cell-cycle and differentiation times must be identical. Third, even if the times were identical, clusters would be structurally unstable. This means that if the cluster is perturbed away from its equilibrium size (for example, a cell dying by apoptosis or injury to the skin, or a cell failing to differentiate), there is no compensatory mechanism to bring it back to its equilibrium size.

Another unlikely possibility is asymmetric division (Watt, 2001). At division, one daughter cell remains a stem cell, whereas the other daughter differentiates into a transit-amplifying cell. This scheme also suffers from being structurally unstable, as discussed in Janes et al. (2002).

A more realistic solution for cluster size stability is a mechanism that actively controls the size via regulation of either the division or differentiation rates. For example, suppose that the division rate is constant with a variable rate of differentiation. Then, if the cluster is below its equilibrium size, there is little or no differentiation. Because of cell division, the cluster will grow. If the cluster is above its equilibrium size, differentiation is upregulated or switched on. If the differentiation rate is fast enough to counteract cell division, the cluster will shrink. These solutions do not suffer from any of the problems mentioned in the last paragraph.

The experiments on the Notch signalling pathway are suggestive of a regulated differentiation rate. However, we can investigate both division and differentiation rate regulation by simulating an appropriate model of stem cell clusters. The model, in this case, is a simplified, two-dimensional representation of cells in the basal layer.

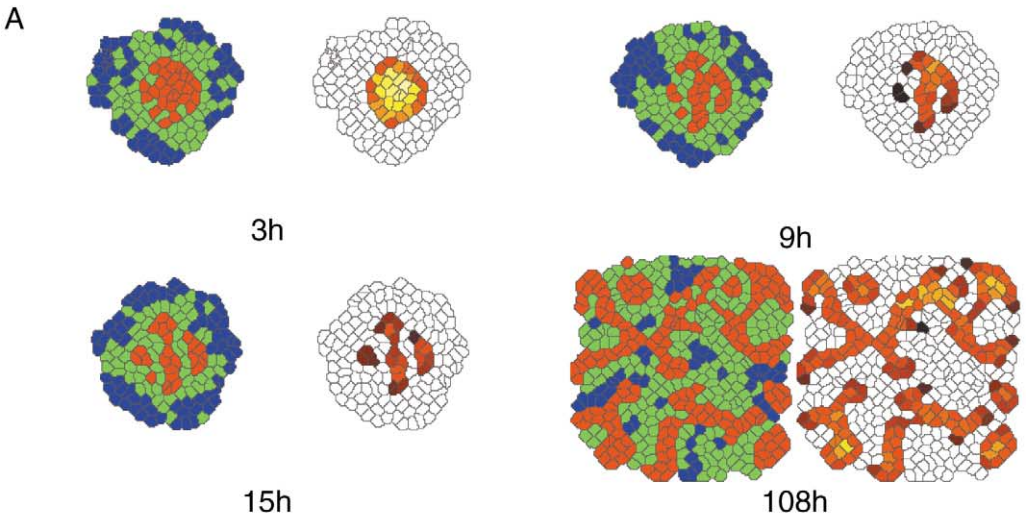
Control via regulation of differentiation

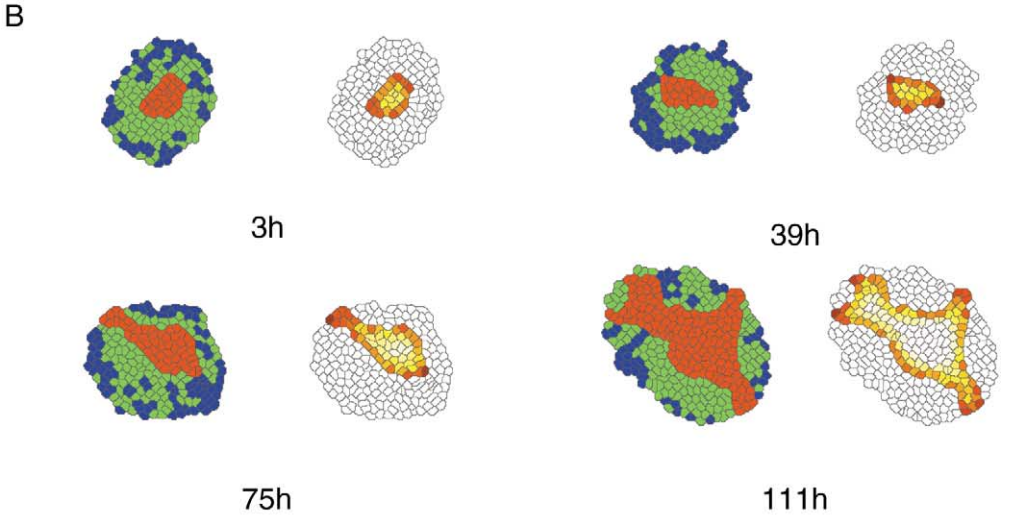
In this section, we model active cluster size control by regulating the differentiation rate. We are not concerned with the biochemical mechanism that causes differentiation of stem cells. Therefore, we impose a maximum cluster size of 30 cells. Only cells on the periphery of the cluster are allowed to differentiate. When the number of cells in the cluster is below the threshold of 30 cells, there is no differentiation. When the threshold is reached, peripheral stem cells immediately differentiate into transit-amplifying cells.

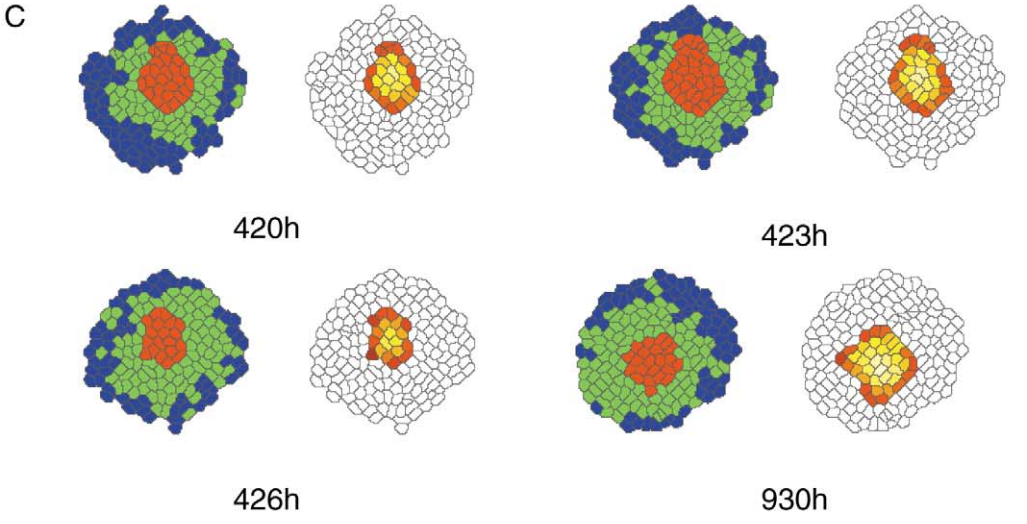
Fig. 1A shows a simulation of a stable cluster supplying the epidermis with differentiated cells. In the first frame (48 h), a cell in the cluster is just about to divide. This will take the number of cells in the cluster to 30 (51 h). This causes differentiation of all peripheral stem cells into transit-amplifying cells (54 h). The cluster now contains 14 cells. The cluster continually grows and shrinks supplying the epidermis with differentiated cells.

There are only two necessary conditions for creating a stable stem cell cluster. The first is trivial: only peripheral stem cells must differentiate. The simulation in Fig. 1B demonstrates what happens when interior stem cells differentiate. When the cluster size reaches threshold, any cell can differentiate, bringing the size back below threshold. The cluster fills with transit-amplifying cells,

Fig. 4. Cluster control using lateral-induction mediated by Notch signalling. The left of each figure shows the cell type, that is, stem (red), transit-amplifying (green), and committed (blue). The right of each figure shows the Notch activation of each stem cell in a cluster. The brighter the colour, the higher the activation. Differentiated cells are coloured white. (A) Peripheral stems cell differentiate when the Notch activation increases above 0.075. This fails because when a peripheral cell differentiates it causes interior cells, with high Notch activation, to become peripheral (3 h). Because these cells have high activation, they differentiate (9 h), causing the cluster to fragment (15 h), leading to uncontrolled growth (108 h). (B) A second condition is imposed to overcome the problem in (A). Peripheral cells with a Notch activation above 0.045 and with more than 15 surfaces in contact with differentiated cells can differentiate. This does not work because the distribution of Notch activation with cluster size (Fig. 2) is not invariant with cluster shape (see Fig. 5). Peripheral cells on the extremities of a cluster have a Notch activation of peripheral cells of a smaller, circular cluster. Therefore, these cells are less likely to differentiate causing the cluster to grow fingers (75 h), leading to uncontrolled growth of the cluster (111 h). This simulation suggests that cluster size control can not depend only on local positional information from the lateral-induction mechanism. (C) A secondary, cluster wide (global) signal is needed to stabilise the cluster. When the Notch activation of any cell in the cluster reaches 0.125, it signals all cells in the cluster to differentiate. Any cell with a Delta expression above 0.075 is deaf to the differentiation signal. This causes peripheral cells at the extremities of the cluster to differentiate, thus making the circular shape stable. The cluster has remained stable for over 900 h.


A
3h
9h
15h
108h


B
3h
39h
75h
111h


C
420h
423h
426h
930h

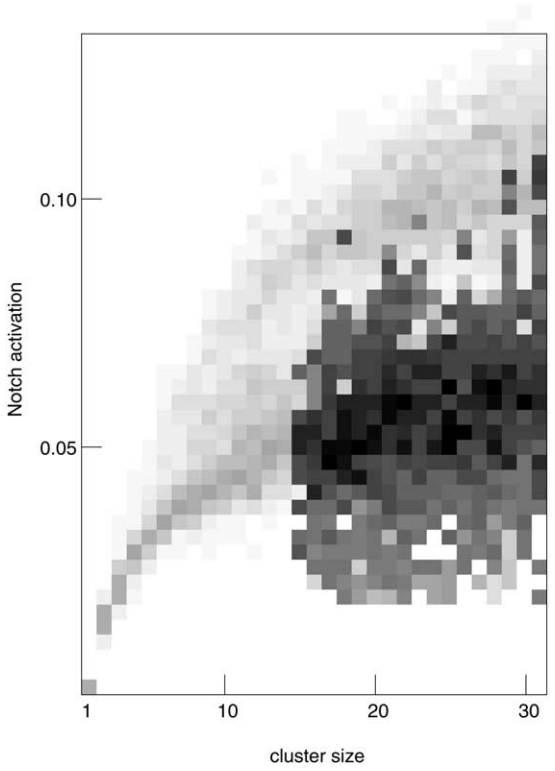


Fig. 5. The distribution of Notch activation against cluster size is not invariant with cluster shape. The distribution of Notch activation with cluster size for the peripheral stem cells in Fig. 4B (dark) is superimposed on the distribution for a circular cluster (light), shown in Fig. 2.

shown in the first two frames (12–27 h), until it fragments (30 h). The two new clusters also fragment, leading to uncontrolled growth, as shown in the last frame. The second condition is that stem cells must adhere more strongly to each other than they do to transit-amplifying and committed cells. This is demonstrated in the simulation in Fig. 1C. The cluster loses its rounded shape. Cells on the periphery are not pulled into the main cluster body. The result is that stem cells can break away from the cluster, or, when differentiation occurs, multiple clusters are formed (69 h). This is a runaway process leading to uncontrolled growth.

Control via regulation of division

For this case, peripheral stem cells are continuously differentiating, whatever the size of the cluster. When the cluster is below threshold, all cells are in the cell-cycle. When the cluster is above threshold, all cells are assumed to be in a resting G_0 state. In the last section, all peripheral cells differentiate immediately when the threshold was reached. Now, we cannot have all peripheral cells differentiating, otherwise the cluster would rapidly disappear. Therefore, we assume that there is a small probability per unit time p, that a peripheral cell will differentiate. In Fig. 1D we simulate this idea with $p = 0.05\ h^{-1}$ The cluster fragments (21 h) because differentiation causes cells deep inside the cluster to become peripheral.

We can solve this problem by only selecting those cells with the fewest surface contacts to other stem cells. These cells are positioned on the outer edge of the cluster and so do not include the peripheral cells that differentiated in Fig. 1D. In Fig. 1E, we show a simulation (with $p = 0.1$) where peripheral cells differentiate only if they have less than 23 surface contacts with other stem cells. The solution works. However, it is not very robust to changes in p or the number of surface contacts. If we select cells with slightly too many contacts (for example, 25), then the cluster fragments as in Fig. 1D (result not shown). If we select cells with slightly too few contacts (for example, 20) or if p is slightly too small (0.08), then there is not enough differentiation to maintain the transit-amplifying and committed cells. If p is slightly too high (1.2), then the differentiation rate is too fast and the cluster disappears.

Thus, control via regulation of division is much harder to achieve than control via differentiation. In the former case, the differentiation rate has to be balanced precisely. It has to be high enough to supply differentiated cells to the epidermis, but not too high so as to cause the cluster to disappear. In the latter case, however, there are no conflicting requirements: the differentiation rate need only be faster than the division rate.

Given that the experimental data also point to regulation of differentiation, for the rest of this paper, we focus on this mechanism.

Summary

The general rules for active cluster size control via regulation of differentiation can be summarised as follows.

1. Some or all cells in the cluster divide.
2. If the number of cells in a cluster is above equilibrium, cells on the periphery of the cluster differentiate into transit-amplifying cells.
3. The average time taken to differentiate must be shorter than the average cell-cycle time.
4. Stem cells must adhere more strongly to each other than they do to differentiated cells.

Rule 1 is self-evident. Rule 2 has been observed indirectly by overexpression of Delta (Lowell et al., 2000). There is no evidence in vivo for rule 3. However, division of cells in culture occurs on average every 16 h (Dover and Potten, 1988), and cells in suspension are known to be irreversibly committed to differentiation after 12 h (Adams and Watt, 1989). There is no direct evidence for rule 4. However, stems cells are known to be more adhesive to ECM than transit-amplifying cells, and cell–cell and cell–ECM adhesiveness are not totally independent (Hodivala and Watt, 1994; Jensen et al., 1999).

The Notch signalling pathway can mediate cluster size

Theoretical considerations

In the last section, we imposed an artificial maximum cluster size of 30 cells. We now consider how the cluster can autonomously initiate differentiation when it becomes too large. As discussed in the Introduction, the experimental evidence points to a role for the Notch signalling pathway.

The data suggest that high expression of Delta and high activation of Notch are necessary for stem cell differentiation. Our previous arguments about active cluster size control lead us to propose the following hypothesis. When a stem cell cluster is small, Delta expression is low in all cells of the cluster. This means that Notch activation is low and there is no differentiation. The cluster grows. When the cluster is too large, Delta expression is high, Notch activation is high, and cells are signalled to differentiate. The cluster shrinks. This hypothesis is not the whole solution because we have not answered the following three questions. First, how does the size of the cluster affect Delta expression and Notch activation? Second, why does high Delta expression cause some cells to differentiate and others not. And third, how are peripheral and interior stem cells distinguished so that only peripheral cells differentiate?

We require a mechanism that causes Delta expression to increase in all stem cells in a cluster as the number of these cells increases. This could occur via an additional diffusible regulator. But a more natural explanation is the lateral-induction mode of Delta-Notch signalling. This is where activated receptor upregulates the production of its own ligand. Neighbouring cells stimulate each other to produce high levels of ligand and hence high levels of receptor activation. A consequence of this effect is that the more cells that participate in lateral-induction, the higher the levels of receptor activation and ligand expression in all cells, which is precisely the phenomenon we require.

Lateral-induction has already been shown to be mediated by Delta-Notch signalling (de Celis and Bray, 1997; Huppert et al., 1997; Lewis, 1998). We propose that active control of stem cell cluster size can be achieved by lateral-induction mediated by the Notch signalling pathway. Thus, the Notch signalling pathway may not only be used to indicate cluster size, it may also be used to control cluster size. We now extend our model to test this mechanism.

The Model of Notch mediated lateral-induction

We first consider the model of Notch mediated lateral-induction. Our proposed model is based on previous models of lateral-induction (Owen et al., 1999, 2000; Wearing et al., 2000), which, in turn, are based on a model of epidermal growth factor binding to its receptor (Waters et al., 1990). We calculate the total Delta expressed and the total bound Notch receptor on a cell's membrane. We assume that the Notch activation in a cell is proportional to the total bound receptor. Cell fate is determined by Notch activation and total Delta expression.

Before we simulate Notch-mediated cluster size control, it is instructive to study some of the properties of the lateral-induction model.

Even though this model has five independent parameters, it appears to have only one mode of behaviour. As the number of cells in a cluster increases, the average Notch activation in all cells increases. In Fig. 2, we plot the distribution of Notch activation in the cells against cluster size. At small cluster sizes, there are only peripheral cells. Their Notch activation increases as the cluster size increases. This occurs because, as additional cells are added to the cluster, they increase the Notch activation in their neighbouring cells. These cells produce more Delta which, in turn, increases the Notch activation in their neighbours, and so on.

At even larger sizes, the cluster contains interior cells. These cells have a larger Notch activation than peripheral cells because they are completely surrounded by stem cells. Part of the surfaces of peripheral cells contact transit-amplifying cells, and hence they have lower Notch activation. After a certain cluster size, Notch activation saturates. The distribution of Notch activation is bimodal, reflecting the different Notch activations of peripheral and interior cells. Thus, lateral-induction can distinguish between these two groups of cells.

The absolute Notch activation and the cluster sizes above which Notch activation saturates depend on the particular parameter values chosen (results not shown). However, the qualitative behaviour of the model is invariant with the actual parameter set we choose.

In Fig. 3A, we plot total Delta expression against Notch activation in a cell. The two are highly correlated as expected. In Fig. 3B, we plot Notch activation for each cell against the number of surfaces each cell has in contact with other stem cells. Interior cells are, by definition, surrounded by stem cells and have the highest Notch activation due to their many surface contacts. Peripheral cells contact stem and differentiated cells, so have fewer stem cell surface contacts and, hence, lower activation.

So, Notch-mediated lateral-induction appears to give us an autonomous mechanism able to signal to cells the size of the cluster they are in and their position within the cluster. Stem cell differentiation occurs when the Notch activation in a cell is high. Let us postulate a threshold level of Notch activation, above which a cell is signalled to differentiate.

We are now ready to simulate Delta-Notch control of cluster size. However, as we discuss below, there are still some problems to solve. We show that, as it stands, the model is too simple and we need to postulate further mechanisms to make it work.

Control cannot be mediated by local positional information

In this section, we will show that cells relying only on their Notch activation to determine their fate cannot form

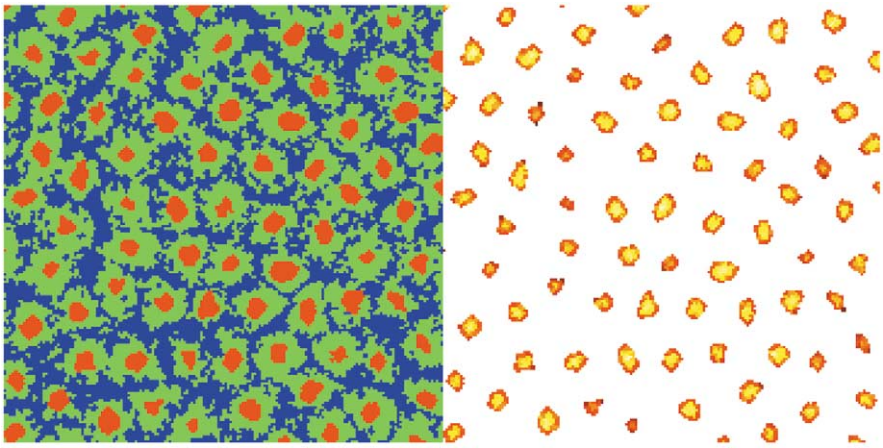


Fig. 6. Multiple clusters in a simulated 1-mm^2 area of basal epidermis. All clusters have remained stable and fixed relative to their neighbours for over 1000 h. The image is cropped to hide the effects of the boundary conditions, which are not important.

stable clusters. We do this in several steps, increasing the complexity of the model at each stage.

If differentiation is induced by high Notch activation, we immediately see a problem. If the cluster is growing in size, interior cells are the first to experience the Notch-activation threshold. Therefore, these cells will differentiate before peripheral cells. To overcome this problem, we might postulate an additional signal that, in combination with the Notch signal, only causes peripheral cells to differentiate. For the purpose of illustration, we do not need to specify the biochemical details of this signal. We assume, therefore, that any peripheral cell that reaches the Notch-activation threshold differentiates.

We simulate this idea in Fig. 4A. It is clear that it does not work. This is because, when a peripheral cell differentiates, its neighbouring interior cells become peripheral cells (e.g., 3 h). These cells have a large number of surface contacts with stem cells and hence a Notch activation above threshold. These cells differentiate (9 h) and the cluster fragments.

We can solve this problem by selecting only those cells with the fewest surface contacts to other stem cells. These cells are positioned on the outer edge of the cluster, and so do not include the peripheral cells positioned deep within the cluster that differentiated in Fig. 4A. They are also the cells with the lowest Notch activation. In Fig. 4B, we simulate differentiation of peripheral cells with more than 15 surfaces in contact with differentiated cells and a Notch activation above threshold. Now the correct cells differentiate, but over the long term the cluster loses its circular shape and grows fingers. This happens because the distribution of Notch activation with cluster size (Fig. 2) is not invariant with cluster shape. In Fig. 5, we plot the distribution of Notch activation of peripheral cells from the simulation in Fig. 4B (shown darkened) and superimpose it on the distribution of Notch activation of cells from a circular cluster, shown in Fig. 2 (shown lightened). Peripheral cells in a finger-shaped cluster have a Notch activation of peripheral cells of a smaller, circular cluster. Therefore, these cells are less likely to differentiate, causing the cluster to grow fingers (75 h). This is a runaway process leading to uncontrolled growth of the cluster (111 h).

On the basis of these results, we conclude that stem cell fate cannot depend on local positional information using Notch-mediated lateral induction. Therefore, we have to either abandon lateral-induction or postulate a cluster-wide (global) secondary signal. This signal should act in conjunction with the Notch signal to initiate differentiation.

Control can be mediated by a secondary global signal

A natural possibility for a secondary signal is that when the Notch activation of any cell in the cluster reaches threshold, it sends a signal to all cells in the cluster to differentiate. What is there to stop interior cells from differentiating if there is a global signal to differentiate? Fortunately, we already have a solution to this problem. Interior cells have significantly higher Delta expression levels than peripheral cells, and cells with high Delta expression are known to be deaf to the differentiation signal (Lowell et al., 2000). Therefore, by selecting an appropriate threshold level for Delta expression, we can make sure that only peripheral cells differentiate. The nature of the global signal is open to speculation. Two examples could be a pulse of Ca^{2+} or cAMP that propagate from the signalling cell through the cluster in a wave. A cell receiving this pulse and having Delta expression below threshold will be signalled to differentiate.

We can simulate this idea without having to consider the actual biochemical details of this global secondary signal. When any cell in the cluster reaches a threshold level of Notch activation, we assume that a differentiation signal is sent to all cells in the cluster. Any cell with sufficiently high Delta expression is deaf to this signal. The Delta expression threshold should not be too small, otherwise no cells will differentiate, or too large, otherwise all cells will differentiate.

Fig. 4C shows a typical simulation in which the shape and size of the cluster remains stable. Because cells with a sufficiently high Delta expression are deaf to the differentiation signal, only peripheral cells that are on the extremities of the cluster differentiate. This causes the circular shape to be stable.

Finally, to show that this idea works for multiple clusters, we simulate a 1-mm^2 area of basal epidermis (Fig. 6). The clusters remain in their relative positions because of the radial force of cell flux from the clusters. In this simulation, the boundary conditions are not important. However, we can use different boundary conditions to simulate different biological scenarios. For example, periodic boundaries can simulate unbroken, normal epidermis, no flux boundaries can simulate the edges of a petri dish.

Conclusion

Recent experimental advances in the identification of epidermal stem cells have shown that the basal layer of interfollicular epidermis is highly structured. Clusters of stem cells provide a continuous supply of differentiated cells, which gradually move into the suprabasal layers. In this paper, we have focused on the way in which a stem cell cluster can maintain its size and shape in such a dynamic environment. We argue that control of the cluster shape requires, simply, that stem cells adhere to each other more strongly than to differentiated cells. Size control is more complex, requiring differentiation on the edge of the cluster to be controlled by cluster size. We have shown that this can be achieved via Notch-mediated lateral-induction, which is consistent with experimental evidence linking Notch activation and Delta expression with stem cell differentiation. We argue that Notch signalling may have a dual role. First, the mechanism of Notch mediated lateral-induction gives cells information on their position in, and the size of, the cluster they inhabit. Second, the high levels of Delta expression and Notch activation cause cell differentiation and hence control cluster size. We show that the control of differentiation depends both on the level of Delta expression and on a secondary global signal that is produced in response to Notch activation.

Our work has focused on a single stem cell cluster. An important phenomenon not addressed by the model is the ability of the basal layer of the epidermis to reconstitute itself from isolated stem cells, with a single stem cell giving rise to multiple stable clusters (Jones et al., 1995). In its current form, the model is unable to reproduce this phenomenon, which would require special properties for the cells at the edge of the growing colony. Preliminary investigations show that amending the model to include this does enable simulation of multiple cluster formation, and further study of this is a natural area for future work. Another behaviour worth exploring with a multicluster model is clonal expansion of mutant cells. Zhang et al. (2001) have shown that EPUs contain the expansion of p53-mutant clones of neighbouring EPUs. Colonisation of mutant clones into adjacent EPUs only occurs under sustained carcinogenic exposure. A model may be able to discover necessary conditions for containment and colonisation.

Another unanswered question concerns the three-dimensional structure of the epidermis. Stem cell clusters lie either at the tips of the dermal papillae or at the bottom of rete pegs, depending on site in the body and possibly regulated by epidermal thickness. Investigation of this is a natural area for future work using a three-dimensional version of the model. This will also allow us to explore the significance of differential adhesion and motility on ECM between stem and transit-amplifying cells (Jensen et al., 1999). For example, differential adhesion to the basement membrane might cause stable clusters without the need to invoke increased cell–cell adhesion between stem cells.

A third potential application for the model is in psoriasis. This commonly occurring skin disease is characterised by red scaly lesions, and the whole structure of the epidermis is disrupted, with many dividing cells in the suprabasal layers, highly elongated rete pegs, and incomplete epidermal differentiation. Preliminary evidence suggests that the Notch signalling pathway may be disrupted in psoriasis (Moran et al., 1999), and further experimental data in this area would enable the model to be extended to psoriatic epidermis.

Acknowledgments

N.J.S. and J.A.S. were supported by SHEFC Research and Development Grant 107 “Centre for Theoretical Modelling in Medicine.” J.A.S. was supported, in part, by an Advanced Research Fellowship from EPSRC.

References

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 α-5-β-1-integrin loss from the cell-surface. Cell 63, 425–435.

Arnold, I., Watt, F.M., 2001. c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. Curr. Biol. 11, 558–568.

Artavanis-Tsakonas, S., Rand, M.D., Lake, R.J., 1999. Notch signalling: cell fate control and signal integration in development. Science 284, 770–776.

Asplund, A., Guo, Z., Hu, X., Wassberg, C., Pontén, F., 2001. Mosaic pattern of maternal and paternal keratinocyte clones in normal human epidermis revealed by analysis of X-chromosome inactivation. J. Invest. Dermatol. 117, 128–131.

Baron, M., Aslam, H., Flasza, M., Fostier, M., Higgs, J.E., Mazaleyrat, S.L., Wilkin, M.B., 2002. Mutiple levels of Notch signal regulation. Mol. Membr. Biol. 19, 27–38 (Review).

Barrandon, Y., Green, H., 1985. Cell size as a determinant of the clone forming ability of human keratinocytes. Proc. Natl. Acad. Sci. USA 82, 5390–5394.

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

Brakebusch, C., Grose, R., Quondamatteo, F., Ramirez, A., Jorcano, J.L., Pirro, A., Svensson, M., Herken, R., Sasaki, T., Timpl, R., Werner, S., Fassler, R., 2000. Skin and hair follicle integrity is crucially dependent on β-1 integrin expression on keratinocytes. EMBO 19, 3990–4003.

Bray, S., 1998. Notch signalling in *Drosophila*: three ways to use a pathway. Semin. Cell Dev. Biol. 9, 591–597.

de Celis, J.F., Bray, S., 1997. Feedback mechanisms affecting Notch activation at the dorsoventral boundary in the *Drosophila* wing. Development 124, 3241–3251.

Dellambra, E., Golisano, O., Bondanza, S., Siviero, E., Lacal, P., Molinari, M., D'Atri, S., De Luca, M., 2000. Downregulation of 14-3-3σ prevents clonal evolution and leads to immortalization of primary human keratinocytes. J. Cell Biol. 149, 1117–1130.

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

Dover, R., Potten, C.S., 1988. Heterogeneity and cell cycle analyses from time-lapse studies of human keratinocytes in vitro. J. Cell Sci. 89, 359–364.

Dover, R., Watt, F.M., 1987. Measurement of the rate of epidermal terminal differentiation: expression of involucrin by S-phase keratinocytes in culture and in psoriatic plaques. J. Invest. Dermatol. 89, 349–352.

Dubertret, B., Rivier, N., 1997. The renewal of the epidermis: a topological mechanism. Biophys. J. 73, 38–44.

Duffill, M., Wright, N.A., Shuster, S., 1976. The cell proliferation kinetics of psoriasis examined by three in vivo techniques. Br. J. Dermatol. 94, 355–362.

Fuchs, E., 1990. Epidermal differentiation: the bare essentials. J. Cell Biol. 111, 2807–2814.

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

Graner, F., Glazier, J.A., 1992. Simulation of biological cell sorting using a 2-dimensional extended potts-model. Phys. Rev. Lett. 69, 2013–2016.

Graner, F., Glazier, J.A., 1993. Simulation of the differential adhesion driven rearrangement of biological cells. Phys. Rev. E 47, 2128–2154.

Hodivala, K.J., Watt, F.M., 1994. Evidence that cadherins play a role in the downregulation of integrin expression that occurs during keratinocyte 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.

Huppert, S.S., Jacobsen, T.L., Muskavitch, M.A.T., 1997. Feedback regulation is central to Delta-Notch signalling required for *Drosophila* wing vein morphogenesis. Development 124, 3283–3291.

Iizuka, H., Ishida-Yamamoto, A., Honda, H., 1996. Epidermal remodelling in psoriasis. Br. J. Dermatol. 135, 433–438.

Janes, S.M., Lowell, S., Hutter, C., 2002. Epidermal stem cells. J. Pathol. 197, 479–491.

Jensen, U.B., Lowell, S., Watt, F.M., 1999. The spatial relationship between stem cells and their progeny in the basal layer of human epidermis: a new view based on whole-mount labelling and lineage analysis. Development 126, 2409–2418.

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. Natl. Acad. Sci. USA 93, 14025–14029.

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

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.

Kaufman, C.K., Fuchs, E., 2000. It's got you covered. NF-κb in the epidermis. J. Cell Biol. 149, 999–1004.

Laporte, M., Galand, P., Fokan, D., de Graef, C., Heenen, M., 2000. Apoptosis in established and healing psoriasis. Dermatology 200, 314–316.

Levy, L., Broad, S., Diekmann, D., Evans, R.D., Watt, F.M., 2000. β-1 integrins regulate keratinocyte adhesion and differentiation by distinct mechanisms. Mol. Cell. Biol. 11, 453–466.

Lewis, J., 1996. Neurogenic genes and vertebrate neurogenesis. Curr. Opin. Neurobiol. 6, 3–10.

Lewis, J., 1998. Notch signalling: a short cut to the nucleus. Nature 393, 304–305.

Loeffler, M., Potten, C.S., Wichmann, H.E., 1987. Epidermal cell proliferation. II. A comprehensive mathematical model of cell proliferation and migration in the basal layer predicts some unusual properties of epidermal stem cells. Virchows Arch. B 53, 286–300.

Lowell, S., Jones, P., Le Roux, I., Dunne, J., Watt, F.M., 2000. Stimulation of human epidermal differentiation by Delta-Notch signalling at the boundaries of stem-cell clusters. Curr. Biol. 10, 491–500.

Lowell, S., Watt, F.M., 2001. Delta regulates keratinocyte spreading and motility independently of differentiation. Mech. Dev. 107, 133–140.

Marée, A.F.M., Panfilov, A.V., Hogeweg, P., 1999. Phototaxis during the slug stage of *Dictyostelium discoideum* slugs: a model study. Proc. R. Soc. Lond. B Biol. Sci. 266, 1351–1360.

Moran, J.L., Johnston, S.H., Rauskolb, C., Bhalerao, J., Bowcock, A.M., Vogt, T.F., 1999. Genomic structure, mapping, and expression analysis of the mammalian Lunatic, Manic, and Radical fringe genes. Mamm. Genome. 10, 535–541.

Oro, A.E., Scott, M.P., 1998. Splitting hairs: dissecting roles of signalling systems in epidermal development. Cell 95, 575–578.

Owen, M.R., Sherratt, J.A., Myers, S.R., 1999. How far can a juxtacrine signal travel. Proc. R. Soc. Lond. B Biol. Sci. 266, 579–585.

Owen, M.R., Sherratt, J.A., Wearing, H.J., 2000. Lateral induction by juxtacrine signalling is a new mechanism for pattern formation. Dev. Biol. 217, 54–61.

Potten, C.S., 1974. The epidermal proliferative unit: the possible role of the central basal cell. Cell Tissue Kinet. 7, 77–84.

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

Potten, C.S., Wichmann, H.E., Loeffler, M., Dobek, K., Major, D., 1982. Evidence for discrete cell kinetic populations in mouse epidermis based on mathematical analysis. Cell Tissue Kinet. 15, 305–329.

Raghavan, S., Bauer, C., Mundschau, G., Li, Q., Fuchs, E., 2000. Conditional ablation of β-1 integrin in skin: severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination. J. Cell Biol. 150, 1149–1160.

Rangarajan, A., Talora, C., Okuyama, R., Nicolas, M., Mammucari, C., Oh, H., Aster, J.C., Krishna, S., Metzger, D., Chambon, P., Miele, L., Aguet, M., Radtke, F., Dotto, G.P., 2001. Notch signalling is a direct determinant of keratinocyte growth arrest and entry into differentiation. EMBO J. 20, 3427–3436.

Ren, Z.P., Ahmadian, A., Ponten, F., Nister, M., Berg, C., Lundeberg, J., Uhlen, M., Ponten, J., 1997. Benign clonal keratinocyte patches with p53 mutations show no genetic link to synchronous squamous cell precancer or cancer in human skin. Am. J. Pathol. 150, 1791–1803.

Savill, N.J., 2003. Mathematical models of hierarchically structured cell populations under equilibrium with application to the epidermis. Cell Prolif. 36, 1–26.

Savill, N.J., Hogeweg, P., 1997. Modeling morphogenesis: from single cells to crawling slugs. J. Theor. Biol. 184, 229–235.

Stott, E.L., Britton, N.F., Glazier, J.A., Zajac, M., 1999. Stochastic simulation of benign avascular tumour growth using the Potts model. Math. Comp. Model. 30, 183–198.

Turner, S., Sherratt, J.A., 2002. Intercellular adhesion and cancer invasion: a discrete simulation using the extended Potts model. J. Theor. Biol. 216, 85–100.

Vasioukhin, V., Bauer, C., Degenstein, L., Wise, B., Fuchs, E., 2001. Hyperproliferation and defects in epithelial polarity upon conditional ablation of α-catenin in skin. Cell 104, 605–617.

Waters, C.M., Oberg, K.C., Carpenter, G., Overholser, K.A., 1990. Rate constants for binding, dissociation, and internalization of EGF: effect of receptor occupancy and ligand concentration. Biochemistry 29, 3563–3569.

Watt, F.M., 2001. Stem cell fate and patterning in mammalian epidermis. Curr. Opin. Genet. Dev. 11, 410–417.

Wearing, H.J., Owen, M.R., Sherratt, J.A., 2000. Mathematical modelling of juxtacrine patterning. Bull. Math. Biol. 62, 293–300.

Zhang, W., Remenyik, E., Zelterman, D., Brash, D.E., Wikonkal, N.M., 2001. Escaping the stem cell compartment: sustained UVB exposure allows p53-mutant keratinocytes to colonize adjacent epidermal proliferating units without incurring additional mutations. Proc. Natl. Acad. Sci. USA 98, 13948–13953.

Zhu, A.J., Haase, I., Watt, F.M., 1999. Signaling via β-1 integrins and mitogen-activated protein kinase determines human epidermal stem cell fate in vitro. Proc. Natl. Acad. Sci. USA 96, 6728–6733.

Zhu, A.J., Watt, F.M., 1999. β-Catenin signalling modulates proliferative potential of human epidermal keratinocytes independently of intercellular adhesion. Development 126, 2285–2298.