Communication compartments in hair follicles and their implication in differentiative control

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Summary

Observations on hair follicles presented in this paper show that boundaries to junctional communication are formed between groups of cells following different pathways of differentiation. The patterns of junctional communication in the bulbs of rat vibrissa follicles and human hair follicles were studied by microinjection of the fluorescent tracer dye Lucifer Yellow CH. Dye spread was extensive between undifferentiated cells of the hair bulb matrix but communication boundaries were found between groups of morphologically distinct cells. For example, boundaries to dye spread were observed between undifferentiated matrix cells and cells in the early stage of differentiation into the inner root sheath, between Huxley's and Henle's layers in the early inner root sheath and between cells of the cuticle and cortex of the hair. Dye did not spread between epithelial cells of the hair bulb and mesenchymal cells of the connective tissue sheath or dermal papilla. The patterns of dye spread became more complex (increased boundary formation and subcompartmentation) as differentiation progressed in higher regions of the hair bulb. The observed communication can be related to previous ultrastructural studies by others on the distribution of gap junctions in the wool follicle. These results show that junctional communication, with its consequent intercellular spread of small ions and molecules, is associated with uniformity of expression and behaviour within cell populations and that interruption of communication through the formation of boundaries and communication compartments is temporally and spatially related to the production of subpopulations of cells committed to the expression of different phenotypes.

Key words: communication compartments, gap junctions, hair follicles, dye-injection.

Introduction

Gap junctions, which are found in most animal tissues, allow small cytoplasmic ions and molecules (up to about 1000 M<sub>r</sub>) to pass directly between cells with little or no apparent limitation with respect to charge or chemical nature (for review, see Loewenstein, 1979; Finbow and Pitts, 1981). Since many ions and molecules involved in the intracellular regulation of cellular activities are small enough to pass freely through the junctional channels, it has been suggested that cell-cell communication via gap junctions may be an important means for the intercellular interaction of such intracellular signal networks within a coupled cell population (Sheridan, 1976; Loewenstein, 1979; Warner and Lawrence, 1982; Kam and Pitts, 1988; reviewed by Pitts et al., 1988; Guthrie and Gilula, 1989). More support for this idea emerged recently when it was shown that there are specific and modifiable patterns of junctional communication in skin. For example, studies based on the microinjection of Lucifer Yellow, a low molecular weight fluorescent dye, which spreads from cell to cell via gap junctions, have shown that dermal fibroblasts and keratinocytes communicate selectively with their respective homologous cell types thereby forming different compartments (Kam et al., 1986; Salomon et al., 1988). The epidermis is further divided into small compartments similar in size and distribution to the previously proposed epidermal proliferative units. These compartments create specific cell networks through which pools of small ions and molecules can equilibrate. Increase in communication between some epidermal compartments and their underlying dermal compartment is observed when the epidermis undergoes hyperproliferation (Kam and Pitts, 1988, 1989), with the likely consequence of redistributing compartmental intracellular pools of signal molecules. A model based on these observations has been proposed to


describe the role of junctional communication in the modulation of epidermal proliferative control (Kam and Pitts, 1988; Pitts et al., 1988). On the other hand, decrease of permeability of compartment boundaries is observed in the epidermis of the skin where keratinocytes undergo terminal differentiation (Kam et al., 1986, 1987). Previous experiments in model systems of mixed cell cultures have shown that the expression of recessive phenotypes can be inhibited by neighbouring cells that are expressing dominant phenotypes through the junctional contacts between the two populations (Subak-Sharpe et al., 1969; Cosaro and Migeon, 1977; Sheridan et al., 1979; Mehta et al., 1986). Therefore it is possible that the expression of the differentiated phenotype in vivo is also regulated by changes in communication as in model systems. In this paper, we present the results of a series of experiments that point to a potential role for cell-cell communication in differentiative control, namely, the development of different cell lineages from a population of undifferentiated cells.

Whisker follicles were chosen for this study because of their large size and well-developed stratification of the different cellular layers. The follicle itself is made up of epithelial cells which are arranged in three distinct parts: (1) the hair shaft, which consists of a medulla and cortex surrounded by cuticle cells, (2) the inner root sheath and (3) the outer root sheath (Fig. 1). Cell division is observed primarily in the basal part of the hair bulb where germinative cells give rise to relatively undifferentiated matrix cells and these cells subsequently undergo differentiation to develop into the four layers of the inner root sheath (companion cells, Henle’s layer, Huxley’s layer and cuticle of the inner root sheath) as well as the three layers of the hair shaft (Clarkson, 1896). On the outside of the inner root sheath is the outer root sheath whose cell lineages seem to develop separately from those of the inner root sheath (Coulombe et al., 1989). The base of the hair follicle is in close contact with the dermal papilla (a condensation of mesenchymal cells, which are believed to have a role in regulating the growth cycle of the hair; Jacobson, 1966; Oliver, 1970) which in turn is attached to the connective tissue sheath surrounding the whole follicle.

The hair follicle is an interesting system for studying differentiation, not only because diverse cell types are generated but also because all the different stages associated with the development are represented simultaneously within the same structure. The spatial organization of various cell types is in effect a temporal record of development. The present study relates the processes of differentiation to the changing patterns of junctional communication and provides new information on the cell-cell interactions that are operative during the formation of different cell lineages.

Materials and methods

Adult August rats were obtained from the Animal Supply facilities, Glasgow University. Rats were killed by ether anaesthesia and whisker follicles in anagen (growth phase) were dissected along with minimal amount of surrounding tissues and kept in ice-cold Heps (GIBCO)-buffered Glasgow Modification of Eagle’s Medium containing 10% fetal calf serum (Flow Laboratories, Irvine, Scotland) for up to 2 hours. The whisker follicle is situated in a connective tissue sheath containing blood sinuses which surround the hair and its associated root sheaths. To permit direct injection of dye into the epithelial cells, a part of the connective tissue sheath was sliced away, taking care to disturb as little as possible the hair bulb and dermal papilla. Microinjection of Lucifer Yellow CH (Sigma) and subsequent processing for microscopy were the same as previously published (Kam et al., 1986; Kam and Pitts, 1988, 1989) except that we routinely left the samples in the resin for up to 3 days before polymerization in order to obtain good histological sections. Serial sections were examined on a Leitz Orthoplan microscope equipped with UV (epi-) illumination and phase-contrast optics. Areas of injection were photographed using a Leitz Orthomat camera attached to the microscope on Kodak Ektachrome 400 films. All fluorescence photomicrographs were taken with a fixed exposure time of 2 minutes. 21 rat vibrissa follicles were injected and analysed. 13 anagen hair follicles from human eye-brow tissue discarded at surgery were also injected and processed in the same way.

Results

Fig. 1 illustrates the arrangement of the cell layers in the rat vibrissa follicle in anagen (growth) phase of the hair growth cycle. The movement of low molecular weight fluorescent tracers between cells is dependent on gap junctions (Loewenstein and Kanno, 1964; Gilula et al., 1972). Stewart (1978) introduced Lucifer Yellow as a fluorescent tracer, which has been used to trace

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Fig. 1. Rat vibrissa follicle: phase-contrast photomicrographs of longitudinal (A) and transverse (B) sections of hair follicles embedded in plastic. The equivalent position of B is indicated on A by short arrows. Bars, 100 μm.

Fig. 2. Fluorescence and phase-contrast photomicrographs of a rat follicle which has been iontophotographically injected with Lucifer Yellow CH into a cell in the matrix region. Although the dye has spread to many cells in the epithelial bulb (cells with fluorescent nuclei are detectable in 18 consecutive sections), it does not enter noticeably into the dermal papilla. Bar, 50 μm.

Fig. 3. Fluorescence and phase-contrast photomicrographs of an injection into a cell at the start of the differentiation zone (indicated by arrows) of the rat follicle. The lowest part of this follicle, including the dermal papilla, has been removed prior to injection. Notice on the upper left edge of this section cells begin to acquire the characteristic appearance of the inner root sheath. Bar, 50 μm.

Fig. 4. Fluorescence and phase-contrast photomicrographs of a transverse section of the mid/upper bulb of a rat follicle showing extensive spread of the injected dye among the cuticle cells (identifiable by their elliptical nuclei) and some of the inner root sheath cells (Huxley’s layer). Notice the sharp change of fluorescence intensity at the boundary (arrowheads) between the Huxley’s layer and the Henle’s layer. Bar, 50 μm.
Table 1. Qualitative summary of the different patterns of intercellular spread of Lucifer Yellow after microinjection into cells in various regions of rat whisker or human eye-brow follicles

<table>
<thead>
<tr>
<th>Injection site</th>
<th>No. follicles injected</th>
<th>Zone of dye spread</th>
<th>Extent of dye spread</th>
<th>Boundaries of dye spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat vibrissa</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lower bulb (matrix)</td>
<td>5</td>
<td>lower bulb (matrix)</td>
<td>extensive</td>
<td>matrix/ORS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>matrix/DP</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>undifferentiated/</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>differentiated zones</td>
</tr>
<tr>
<td>mid-bulb, IRS</td>
<td>3</td>
<td>IRS</td>
<td>limited</td>
<td>within IRS</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>IRS/matrix</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>IRS/cortex</td>
</tr>
<tr>
<td>upper bulb, IRS or cuticles</td>
<td>4</td>
<td>Huxley's layer cuticles,</td>
<td>limited</td>
<td>Huxley's/Henle's</td>
</tr>
<tr>
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<td></td>
<td>Henle's Layer,</td>
<td></td>
<td>cuticle/cortex</td>
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<tr>
<td></td>
<td></td>
<td>companion cells</td>
<td></td>
<td>within cuticle</td>
</tr>
<tr>
<td>ORS</td>
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<td>no spread or 2 or 3</td>
<td>within ORS</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>cells only</td>
<td>ORS/CTS</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>ORS/IRS</td>
</tr>
<tr>
<td>CTS</td>
<td>5</td>
<td></td>
<td>no spread</td>
<td></td>
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<tr>
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<td></td>
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<td></td>
</tr>
<tr>
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<td>1</td>
<td>matrix</td>
<td>extensive</td>
<td>matrix/IRS</td>
</tr>
<tr>
<td>stalk of DP</td>
<td>3</td>
<td>Stalk of DP</td>
<td>limited</td>
<td>Stalk of DP/DP</td>
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<td></td>
<td></td>
<td>Stalk/matrix</td>
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<tr>
<td>CTS</td>
<td>8</td>
<td>CTS</td>
<td>no spread</td>
<td></td>
</tr>
</tbody>
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The dye-spread patterns are described in the text and illustrated in Figs 1-9.
Abbreviations: DP, dermal papilla; IRS, inner root sheath; ORS, outer root sheath; CTS, connective tissue sheath.

Pathways of cell-cell communication in skin (Kam et al., 1986; Salomon et al., 1988) and other tissues (Michaels and Sheridan, 1981; Warner and Lawrence, 1982; Kalimi and Lo, 1988; Serras et al., 1989). The patterns of dye spread in rat vibrissa and human hair follicles are described below and summarised in Table 1. In the rat hair follicle, dye injected into a cell in the matrix region spreads to many other matrix cells (Fig. 2) indicating that the lower bulb cells communicate freely with each other. The dye, however, does not spread into the cells of the dermal connective tissue sheath or dermal papilla. In the region where matrix cells start to differentiate into various layers, cells aligned to form the inner root sheath do not appear to communicate with their counterparts in the cuticle (Fig. 3). In the region where the different layers of the hair have become distinguishable (Fig. 4), it appears that cells in Huxley's layer are in the same compartment as the cells of the cuticle and the cortex but those in Henle's layer are not. This partition between Huxley's and Henle's layers is not absolute (Fig. 5) as dye spreads at a low level from Henle's layer to Huxley's layer. However, the different intensities of dye staining in the two layers show that there is a reduction in permeability between them. Although some outer root sheath cells communicate with inner root sheath cells (Fig. 5) others appear

Fig. 5. Fluorescence and phase-contrast photomicrographs of contiguous serial sections of an injection site at the differentiation region of a rat follicle. Notice the dye spreads from a cell at the outer root sheath (solid arrowhead) to the companion cells lying between the outer and inner root sheaths (double arrowhead) and then to cells in the inner root sheath. Although the injected dye stained both the Henle's and Huxley's layers of the inner root sheath, the sharp reduction of fluorescence (open arrowhead) suggests that the two layers are in different communication compartments, an observation that is complementary to that illustrated in Fig. 4. Bar, 50 μm.

Fig. 6. Fluorescence and phase-contrast photomicrographs of an injection into a cell in the outer root sheath of a rat follicle. No dye spread is seen into the inner root sheath. Fluorescence is detectable in only two outer root sheath cells in the 10 sections obtained from this site. Bar, 50 μm.

Fig. 7. Fluorescence and phase-contrast photomicrographs of a transverse section of the upper bulb of an injected rat follicle showing communication compartments in cuticle cells of the hair. The sharp contrast of fluorescence intensity at the front of dye spread (arrow) indicates the existence of communication barriers within the cuticle, between the cuticle and the inner root sheath and between the cuticle and the cortex of the hair. Bar, 50 μm.

Fig. 8. Fluorescence and phase-contrast photomicrographs of a human eye-brow follicle microinjected at the matrix region. The lower part of the follicle, including the dermal papilla, was removed when the surrounding connective tissue sheath was sliced open prior to injection. Notice although the dye spreads to many germinative cells, it only spreads to a few of the cuticle cells of the inner root sheath (arrows). Bar, 50 μm.

Fig. 9. Fluorescence and phase-contrast photomicrographs of an injection site at the base of the dermal papilla of a human eye-brow follicle. The intracellular staining, appears to be localized within a small number of connective tissue cells. Bar, 50 μm.
not to communicate (Fig. 6). In the more differentiated region of the upper bulb, cuticle cells are organized into small communication compartments and are separated from the cortex (Fig. 7).

For comparison, results of injecting human hair follicles are illustrated in Figs 8 and 9. Fig. 8 shows that dye spreads extensively throughout the matrix cells but only weakly into some of the cuticle cells of the inner root sheath. It therefore appears that the rat and human follicles, in these regions at least, are similarly organized. In addition, an injection into the stalk of the dermal papilla showed an absence of dye spread between the cells of the stalk and the dermal papilla (Fig. 9). Similarly, there was no dye spread into the adjacent epithelial cells. It is also worth noting that in Fig. 9 where some dye has leaked into the extracellular space, the extensive extracellular staining is not accompanied by intracellular staining. This is consistent with earlier studies which showed that the injected dye is not distributed from cell to cell via the extracellular space.

Discussion

Cells connected by gap junctions share their pools of small ions (Loewenstein, 1979), metabolites (Finbow and Pitts, 1981) and control molecules (Lawrence et al., 1978, Puschel and Jungermann, 1988). The resulting homeostasis within such communication compartments may make it difficult for individual cells to express different and new phenotypes (Pitts et al., 1988). However, the division of a compartment into subcompartments by the formation of new boundaries allows groups of cells to gain independence and develop distinct phenotypes. Observations reported in the present study support these general ideas in that the undifferentiated cells in the lower hair bulb matrix are all present in one communication compartment and progressive differentiation is associated with, or perhaps dependent on, progressive compartmentalization.

The outer root sheath may be the source of cells that form the germinative matrix in successive growth cycles (Cotsarelis et al., 1990; Reynolds and Jahoda, 1991). It is therefore of interest that dye spread within the outer root sheath and between the outer root sheath and the matrix was extremely limited in contrast to the extensive spread within the matrix itself.

The compartmentalization of communication in hair follicles may be an example of a more general scheme for the generation of polytypic tissues. Recent studies have shown that in developing embryos of the molluscs Lymnaea stagnalis (Van den Biggelaar and Serras, 1988) and Patella vulgata (Serras et al., 1989) and in embryos of the teleost fish Barbus conchonius (Gevers and Timmermans, 1991), communication boundaries progressively appear between groups of cells with different developmental fates. Similarly in the 7.5-day old mouse embryo (Kalimi and Lo, 1988), communication compartments, which can be delineated by dye-injection, contain cells committed to different lineages.

In differentiating cultures of chick limb bud cells, dye transfer becomes restricted to chondrogenic cells (Coelho and Kosher, 1991) and, in insect larvae, dye transfer (but not electrical coupling) within the epidermis is restricted at segmental boundaries (Warner and Lawrence, 1982). The concurrent emergence of communication compartments and morphologically distinct or differentially committed cell lineages in these systems suggests that compartmentation is likely to have an important role in regulating development and may be prerequisite for the expression of cellular differentiation as proposed earlier (Pitts et al., 1988).

The distribution of gap junctions in the sheep wool follicle has been studied by freeze-etch and thin-section electron microscopy (Orwin et al., 1972, 1973). The results of the dye injections into rat vibrissa follicles are broadly in agreement with the ultrastructural studies in that gap junctions are present in those areas where dye spread occurs and the spreads are more extensive among undifferentiated and precortical cells in the lower bulb where more gap junctions are seen. There is also a general correlation between the reduction in gap junctions seen by electron microscopy and the restrictions in communication between different cell layers as differentiation proceeds. However, the restriction of dye spread occurs at a much lower level in the follicle than the loss of observable gap junctions (Orwin et al., 1973). For example, in the wool follicle, gap junctions between cells of Huxley's and Henle's layers in the inner root sheath persist until the final stages of keratinization of Henle's layer but dye spread between these two layers is already restricted at the earliest stages of differentiation. However, while analysis of dye spreads in living tissues provides a direct, functional measure of junctional communication, electron microscopic observations of gap junctions are unable to distinguish between open and closed forms. The data suggest, therefore, that down regulation in this system is accomplished first by channel closure and later by loss of gap junctional structures. In the larval insect epidermis (Warner and Lawrence, 1982) and in mouse embryos (Kalimi and Lo, 1988), restriction of dye spread occurs even between cells that remain electrically coupled. This apparent anomaly may be due to the different sensitivities of the two analytical procedures but it could mean that some form of junctional communication can take place in the absence of dye transfer. As yet, however, there is no indication of the functional significance of such coupling and electrical measurements have not been made in hair follicles.

As hair growth is a cyclical process, it will be of interest in further work to ascertain if changes in junctional communication accompany the transitions between the growing and resting phases of the hair cycle. Similarly, studies at different stages of the cycle are needed to address the question of whether direct cell-cell communication via gap junctions occurs between matrix cells and dermal papilla cells during the initiation or termination of the hair cycle.

We wish to thank L. Melville, G. Talbot and B. McGuire.
for technical assistance and Dr John Pitts and Dr C. A. B. Jahoda for helpful discussion. This work was supported by the Cancer Research Campaign through a grant to Dr John Pitts.

References


