Tissue-specific regulation of junctional communication in the skin of mouse fetuses homozygous for the repeated epilation (Er) mutation

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Summary

Mouse embryos homozygous for the repeated epilation (Er) gene have abnormally developed skin characterised by hyper-proliferation and incomplete differentiation of the epidermis. In this report, we have studied the patterns of junctional communication in the skin of these mutants to see if the loss of control of proliferation/differentiation is associated with any altered patterns of communication. Using the dye-injection technique we have shown that, compared to normal skin, junctional communication among dermal cells of Er/Er mutants is greatly reduced and the frequency of dermal–epidermal communication is, on the other hand, increased. These results support our previously proposed model, which suggests that selective regulation of junctional communication can be a component of proliferative control in a complex tissue.

Key words: gap junctions, junctional communication, repeated epilation, proliferative control, skin, dye injection.

Introduction

Gap junctions provide pathways for direct cell–cell communication in most animal tissues (Finbow, 1982). Coupled cell populations are partial syncytia (termed communication compartments; Pitts and Kam, 1985) in that they share common pools of ions, metabolites and small control molecules. Within any communication compartment this leads to a form of intercellular homeostasis that coordinates cellular activities and produces tissue rather than cell phenotypes. Junctional communication within compartments is also thought to play a role in developmental signalling (Furshpan and Potter, 1968; Weir and Lo, 1982; Warner et al. 1984) and proliferative control (Loewenstein, 1979; Pitts and Finbow, 1986; Trosko et al. 1988; Yamasaki, 1988).

We have recently mapped the patterns of junctional communication in mouse skin to establish the size and distribution of the communication compartments (Kam et al. 1986; Kam et al. 1987; Kam and Pitts, 1988; Pitts et al. 1988b). These studies have shown that the dermis is extensively coupled (dye injected into one cell for 5 min spreads detectably via junctional pathways into from several hundred to more than two thousand other dermal cells). The epidermis, on the other hand, is divided into many small columnar communication compartments, groups of cells coupled to each other but not to cells in adjacent epidermal compartments or to cells in the dermal compartment. These epidermal compartments are similar in size (10–30 cells) and spatial organisation to the proposed epidermal proliferative units (Potten, 1983). The lateral compartment bound-
maintained at the animal house facility of the Beatson Institute by regular mating of wildtype (+/+ ) and heterozygous (Er/+ ) individuals. Matings of mature Er/+ mice were set up and checked for vaginal plugs the next morning. The day when plugs were found was designated as day 0. On day 15 to 19, fetuses were obtained by Caesarian incision.

**Dye injection**

Strips of skin about 1 mm x 3 mm were excised from the fetuses and kept on ice-cold H-buffered Glasgow modification of Eagle’s medium (Flow Laboratories, Irvine, Scotland) with 10% fetal calf serum for no more than 1.5 h before use. Each strip of skin was iontophoretically microinjected once (with up to 20 nA hyper-polarizing pulses at 1 Hz frequency and 500 ms duration for 5 min) with an electrode filled with 4% aqueous Lucifer Yellow CH (Sigma) solution, processed in LR White (London Resin, Woking, England) and sectioned (5 μm thick). Serial sections of the injected areas were examined on a Leitz Orthoplan microscope equipped with phase-contrast condensers and UV epi-illumination. Photomicrographs of the injected areas were taken on HPS films (DIN 28, Ilford) using a Leitz Orthomat camera. All fluorescence photomicrographs were taken at an exposure time of 30 s.

**Results**

The skin of murine fetuses homozygous for the autosomal Er mutation is characterised by uneven hyperproliferation of the epidermis, absence of the stratum corneum, under-differentiation of the stratum granulosum (Salzgeber and Guenet, 1984), defective development of hair follicles and altered patterns of keratin expression (Holbrook et al. 1982; Fisher et al. 1987). Epidermal overgrowth covers the external orifices making the mutation lethal at birth. The mutant fetuses can be identified after 14 days of development by their pupoid appearance (stumpy limbs and short tails). In the adults, heterozygotes develop recurring patches of defective hair growth, often with some symmetry about the proximodistal axis, but it is not always possible to distinguish between heterozygous and wildtype fetuses.

We have mapped the patterns of junctional communication, by microinjection of Lucifer Yellow, in skin samples dissected from 15- to 19-day-old embryos of Er/Er homozygotes and their normal (wildtype or heterozygous) siblings. Dye injected for 5 min into a cell in normal (Fig. 1 A,B) fetal dermis spreads rapidly into a large number of neighbouring cells ( > 400; seen in all dermal injections of 8 skin samples from litter mates of mutant embryos). This extensive dye spread is in agreement with our earlier results (Kam et al. 1986) and with similar studies on human skin (Salomon et al. 1988). In contrast, dye injected in the same way into a cell in Er/Er dermis (Fig. 1 C,D) spreads to only a few (11.6 ± 9.0; mean ± s.d., n = 7) neighbouring cells or (in 2 cases) is entirely retained in the injected cell.

Also in agreement with earlier observations (Kam et al. 1986; Salomon et al. 1988), injections into the epidermis of normal (wildtype or heterozygous) embryos reveal small compartments of 20–30 cells with distinct lateral and basal boundaries (Fig. 2 A,B).

Junctional coupling in Er/Er epidermis (11 skin samples), unlike that in the dermis, is not reduced. Lucifer Yellow spreads into similar-shaped compartments which are the same size or larger (up to 60 cells) than those in normal epidermis. However, in contrast to those in normal skin, most (8/11) of the epidermal injections spread across the epidermal/dermal boundary. These breaches of the basement membrane-associated communication compartment boundary result in dye transfer to a small number (6.1 ± 5.9, n = 8) of dermal cells close beneath the epidermis (Fig. 2 C,D).

Although hairs fail to develop properly in the Er/Er fetuses, hair nodules can be found in the dermis. Injections into these structures results in good coupling among the epithelial cells (Fig. 3 A–D). However, further compartmentation (Fig. 3 C,D), similar to that found in normal follicles (Kam et al. 1986), can also be seen within the more developed hair nodules. As with the epidermal injections, there can be transfer to dermal cells lying close to the dermal–nodule epithelial boundary (Fig. 3 A,B).

**Discussion**

The patterns of junctional communication in normal and Er/Er fetal skin were mapped by analysing intracellular, iontophoretic injections of the fluorescent dye Lucifer Yellow CH, a widely used procedure which has been applied successfully to intact tissues in earlier studies. This reactive, hydrophilic naphthalimide is an ideal tracer. It passes freely through gap junctions, does not detectably cross intact cell membranes and its distribution does not change significantly during subsequent fixation (Stewart, 1978). Analysis of serial sections made through an injection site provides a three-dimensional map of the dye spread. Under these conditions Lucifer Yellow CH preferentially stains the nucleus but also stains the cytoplasm if enough injected dye is present. Extracellular injections can sometimes be distinguished during the process of iontophoresis but can be identified unambiguously (and disregarded for mapping purpose) by the absence of nuclear fluorescence in the sections (Kam et al. 1986). Matrix staining is a less satisfactory distinguishing criterion, as extracellular dye leakage can occur in the tissue during insertion or removal of the micropipette.

Dye injection is the only practical method for mapping functional pathways of junctional communication in a complex tissue like skin. The circuitry is too complex to usefully interpret electrophysiological measurements, and cDNA or antibody probes to junctional channel components cannot distinguish coupled from uncoupled states, and so cannot be used to locate boundaries or map compartments.

The increased incidence of dermal–epidermal coupling is consistent with the recently proposed model for epidermal growth control (Pitts et al. 1988a,b). In the model, which was developed from ideas put forward by Sheridan (1976) and Loewenstein (1979), loss of second messengers from the growth factor-stimulated basal
Junctional communication in Er skin

Fig. 1. Patterns of junctional communication in dermis of Er/Er and normal skin. A and C are fluorescence and B and D are corresponding phase-contrast photomicrographs of representative injection sites. Injected cells are marked by short arrows. (A,B) The pattern of dye spread from an injection into the dermis of the skin of a normal (wildtype or heterozygous) fetus. About 30 nuclei in this section have a detectable level of fluorescence, and dye can be detected in >400 cells through 21 serial sections. The extensive dye spread in the normal skin results in a lower fluorescence intensity per cell (cf. the higher intensity accompanying the much less extensive dye spread in the Er/Er dermis). In the normal skin, injected dye does not enter the epidermis or the hair follicles. (C,D) The pattern of dye spread from an injection into Er/Er dermis. The injected dye spreads to only one other cell, which is in an adjacent section. A small amount of dye has leaked from the electrode during insertion and removal, leaving traces of extracellular staining. Sections are 5 μm thick. Bars, 20 μm.

cells, by diffusion into the overlying cells of the epidermal compartment (proliferative unit), reduces the sensitivity of the target cells and provides an inhibitory growth modulation that varies directly with compartment size (skin thickness). An essential component of this model is the breakdown of the dermal–epidermal
compartment boundary soon after the stochastic initiation of the cell cycle in one of the five or six sensitive (committed) basal cells in the compartment. It is proposed that the onset of this dermal-epidermal coupling, soon after initiation of the first cell, reduces the concentrations of second messengers in the basal
cells to subthreshold values by diffusion into the extensively coupled dermal 'sink', thus preventing further unwanted divisions. Subsequent division of the initiated cell and reorganization of the basal layer is accompanied by the repair of the boundary and a return to the starting state. The model predicts, therefore, that...
Fig. 2. The pattern of dye spread in the epithelium of normal and Er/Er skin. (A,B) Fluorescence and phase-contrast images of an injection into the inter-follicular epidermis of normal skin. In this section, about 15 cells have a detectable level of fluorescence, while the complete set of serial sections showed that about 40 cells have dye-labelled nuclei. Although there is extracellular staining, particularly round the suprabasal layer, the distribution of fluorescent nuclei and cytoplasmic granules (in the granular layer) permits unambiguous analysis of the pathways of junctional communication (see Discussion). The injected cell, which is partly in an adjacent section, is marked by a short arrow. (C,D) The pattern of dye spread in Er/Er epidermis. Dye injected into an epidermal cell (in an adjacent section) spreads detectably to about 60 cells (40 are seen here). The injected dye also spreads to about 16 dermal cells in this section. Sections are 5 μm thick. Bars, 20 μm.

Fig. 3. The pattern of dye spread in hair nodules of Er/Er skin. (A,B) Injection into a hair nodule at an early stage of development and situated close to the epidermis. About 25 cells inside the nodule have a detectable level of fluorescence. Notice some injected dye has spread to neighbouring dermal cell nuclei. Extracellular staining is present in the matrix materials surrounding the nodule and in the epidermis. (C,D) Injection into a more developed hair nodule. In this section, dye has spread into only some (about 30) of the cells in the nodule. The discontinuous pattern is indicative of compartmentation within the nodule. The level of fluorescence is too high and too even to identify the injected cells in these injections. Sections are 5 μm thick. Bars, 20 μm.

loss of the dermal 'sink' should result in the increase of committed basal cells. The loss of coupling in the Er/Er mutant dermis reduces the effectiveness of the dermal 'sink' and provides a test for the model. The observed epidermal hyperproliferation is consistent with the prediction, and the increased epidermal–dermal coupling (compared to normal skin) is an expected consequence. It represents the increased but unsuccessful attempts of the epidermal cells to invoke normal control.

The Er locus has been mapped to chromosome 4 in the mouse genome (Salzgeber and Guénét, 1984), but the nature of the gene product is not known. Recently, mutations in a Drosophila clock gene (per) have been shown to affect junctional communication between salivary gland cells (Bargigio et al. 1987). The per gene, which has homologous DNA sequences in mouse (Shin et al. 1985), has been shown to code for a proteoglycan (Reddy et al. 1986). It is of further interest, therefore, that proteoglycans have been shown to stimulate junction formation between hepatocytes in primary culture, possibly in a tissue-specific manner (Spray et al. 1987), and may therefore be necessary to allow normal junction formation. In the case of the Er mutation, the reduction of junctional coupling in the dermis may represent the tissue-specific loss or aberrant expression of functionally related molecules that facilitate gap junction formation or are required to stabilize the coupled state.

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References


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