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# Junctional communication and cellular differentiation

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**Summary** Gap junctions provide pathways of direct cell to cell communication in the tissues of metazoan animals. Cells joined by gap junctions share their small ions and molecules but can maintain distinctive activities through expression of different macromolecules which are too large to pass through the junctions. The junctional channels are made of a tissue invariant, evolutionarily conserved 16–18 k protein but the formation and maintenance of active coupling also requires one or more connexins, a family of tissue-specific proteins ranging in size from 21 k to 70 k. Junctions can be isolated as complexes containing both types of protein by mild procedures using high pH but the connexins can be removed by detergent, urea and protease treatment without destroying the characteristic junctional morphology of hexagonally packed channels in the double membrane structures. There is also some evidence for the participation in the complex of tissue-specific proteoglycans which perhaps interact with the tissue-specific connexins and account for specificity of junction formation. Such specificity in mixed cultures leads to the production of communication compartments, groups of cells joined by junctions but separated by reduced trans-boundary coupling from cells in adjacent compartments. Compartmentation also occurs *in vivo* resulting in specific patterns of junctional communication which have been mapped in most detail in mouse skin. These mapping data and the changes which are associated with abnormal proliferation have lead to new ideas on intercellular control. In any compartment where only a proportion of the cells responds to some systemic stimulus (e.g., growth factor) which affects activity through second messengers, homeostatic pressure through loss of second messengers into the non-responding cells should produce a modulating control related to compartment size. Such modulation could provide a growth restraint for producing and maintaining the required cell numbers in different parts of a tissue. Furthermore, as many other cellular activities are also controlled by the concentrations of small cytoplasmic ions and molecules, the emergence of differences in different parts of a developing organism requires compartmentation. Control of the integrity of the compartmental boundaries provides an interesting possible mechanism, again based only on homeostasis (i.e., intercellular equilibration of second messengers or other small ions and molecules) for controlling the expression of difference in the form of, for example, altered growth and/or differentiation.

Gap junctions provide pathways for intercellular communication in the tissues of metazoan animals. They form at points of contact between adjacent cells and provide cytoplasmic continuity through coupled cell populations via sieve-like cell-cell channels. The channels are permeable to small ions and molecules, resulting in extensive intercytoplasmic homeostasis and coordination, but they are not permeable to macromolecules so cells can preserve distinctive characteristics.

Attention has focussed in recent years on the molecular structure of the junctional channel and on the role of junctional communication in the functional organization of cells in tissues. Recent data have provided new insights into junctional structure and function and particular interest now centres on the potential importance of this form of cell-cell interaction in proliferative and developmental signalling.

On the structural side, some recent advances suggest that the number of proteins required to establish and maintain gap junctional communication may be more than hitherto believed. From earlier studies (Makowski *et al.*, 1977; Unwin & Zampighi, 1980) it is known that the gap junction is made from many identical channels, each channel being formed by the end-to-end interaction of two half-channels and each half-channel being composed of six apparently identical protein subunits (Figure 1). As well as the channel forming protein it now seems clear that other molecules are involved in the formation process and in the subsequent stabilization of the gap junctional membrane. These necessary additional elements of the 'junctional complex' appear to be expressed in a tissue-specific manner and can be dissociated from the familiar 'core junction' (Figure 1) during isolation. The multi-component nature of gap junctional communication is consistent with earlier complementation data (MacDonald, 1982), which showed that more than one gene product is required for functional coupling, and can explain the tissue specific loss of coupling seen in mutant organisms (Bargiello *et al.*, 1987; Kam & Pitts, 1988*b*). It provides a basis for understanding the specificity of junction formation seen in culture (Pitts & Bürk, 1976; Fentiman *et al.*, 1976) and a possible mechanism to explain the development of complex, controllable patterns and pathways of junctional communication through tissues *in vivo* (Lo & Gilula, 1979; Warner & Lawrence, 1982; Kam *et al.*, 1986; Serras & van den Biggelaar, 1987; Kam & Pitts, 1988*a*; Salomon *et al.*, 1988).

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### *Vertebrate gap junctions*

Active junctional communication appears to require a highly conserved, tissue invariant 16 k protein (Finbow *et al.*, 1983) and one or more connexins, a family of tissue specific proteins which vary in size (21 k to 70 k) but have limited homology at their N-termini (Beyer *et al.*, 1987; Nicholson *et al.*, 1987; Kistler & Bullivant, 1988). There is also some evidence for the involvement of an extracellular matrix component which may also be tissue specific (Spray *et al.*, 1987). The respective roles of the 16 k protein and the connexins have not been fully elucidated but there is structural evidence suggesting the former is a core component of the junctional channels (Finbow *et al.*, 1983, 1985, 1986, 1988) and functional evidence showing that both the 16 k protein and one or more connexins are required for active coupling (Hertzberg *et al.*, 1985; Dahl *et al.*, 1988; Brummer, 1988; Serras *et al.*, 1988).

The 16 k protein is unrelated to the connexins and, unlike the connexins, is present in all gap junction isolates prepared from vertebrate tissues. It is a hydrophobic protein which occupies a protected position in the junctional structure. It is only sensitive to proteolysis after extraction with SDS, under conditions which cause morphological disruption of the junctions (Finbow *et al.*, 1983) and it is not solubilised from the junctional membrane by agents, such as sarkosyl, triton, deoxycholate, 6 M urea and alkali, to which the gap junction structure is resistant. Peptide mapping studies (Buultjens *et*

*al.*, 1988) have shown that the same 16k protein is present in junctions isolated from different tissues (e.g., liver, heart, kidney, brain) and is highly conserved across vertebrate phyla. Recent sequence data have confirmed the hydrophobicity and the high degree of evolutionary conservation of the 16k protein.

In contrast to the junctions and the 16k protein, the connexins are readily degraded during isolation by endogenous proteases and are not present in all gap junction isolates. Connexins are isolated in highest yield when liver plasma membranes are disrupted by high pH without detergent or 6M urea (Hertzberg, 1984). More vigorous isolation procedures, using sarkosyl and urea for example, strip away most of the connexins, leaving only a small fraction (~10%) still associated with the junctions (Hertzberg & Gilula, 1979; Hertzberg, 1984; Finbow *et al.*, 1980). It is not clear yet whether this minor fraction is retained because it is located in some different structure. The connexins can be removed completely from gap junctions by treatment with protease and sarkosyl (Goodenough, 1974) or by extraction with triton, sarkosyl and urea (Finbow *et al.*, 1983), without loss of the characteristic junctional morphology of close-packed, hexagonal arrays of uniform particles. These particles have a diameter of ~8nm and an apparent central pore which is penetrated and hence revealed by negative staining with uranyl acetate but not with phosphotungstic acid. The yield of the 16k protein is similar in preparations made by different methods but it becomes proportionately and markedly more abundant as the connexins are removed from the junctions by the progressively harsher extraction procedures (Hertzberg & Gilula, 1979; Hertzberg, 1984; Finbow *et al.*, 1985, 1988; Willecke *et al.*, 1988).

The high resolution imaging studies which gave rise to the model shown in Figure 1 were based on X-ray diffraction patterns produced from protease treated, detergent extracted junctions (Makowski *et al.*, 1977). SDS-PAGE of these preparations shows two major bands (between 10k and 16k depending on the reference standards) and no detectable connexins (Goodenough, 1974; Pitts & Finbow, 1986; Finbow *et al.*, 1986; N.B. – the term connexin is now reserved for proteins related by sequence homology to the liver 27k connexin and terms 'connexins' A and B used by Goodenough have been discontinued). The upper band is the 16k protein (identified by peptide mapping; Finbow *et al.*, 1986) and the lower band is a 10k sulph-hydryl protein (Goodenough, 1974; Pitts & Finbow, 1986; Finbow *et al.*, 1986) which runs as higher molecular weight multimers in the absence of reducing agents and which is not found in junctional preparations made by other methods. From the diffraction data (see Figure 1) it is clear that the protein remaining in these stripped junctions forms the channels, that it is mostly buried in the membranes and that very little is exposed on the cytoplasmic faces, the only sites accessible to proteases.

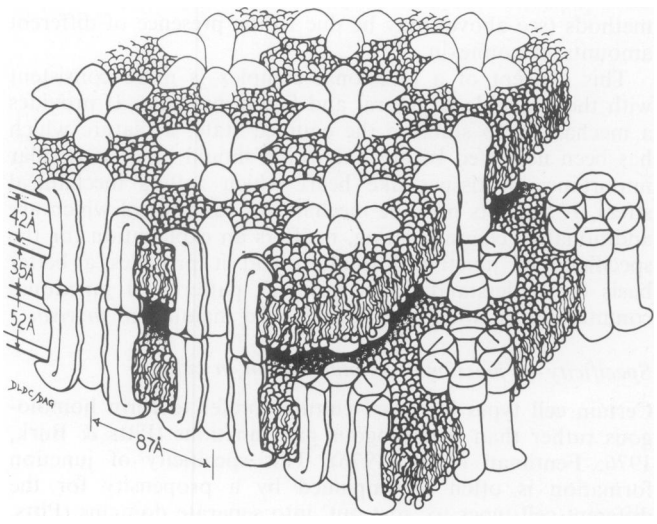


**Figure 1** Model of the gap junction. Reproduced with permission from Makowski *et al.*, 1977.

X-ray diffraction methods, because they analyse junctional pellets, produce average dimensions. Electron diffraction methods (Unwin & Zampigi, 1980; Unwin & Ennis, 1984), on the other hand, use single junctional plaques but have shown that at least some junctions with basically the same dimensions are present in preparations made by other procedures which contain the 16k protein and connexins but no detectable 10k sulphydryl protein.

The presence of the 16k protein in all junction preparations and in particular in the detergent-urea stripped junctions, coupled with its insensitivity to proteolysis while in the intact structures, suggest it is the most likely candidate for the channel protein. The 16k protein however has an apparent molecular weight which is less than the mass of a channel subunit (20,000–30,000 daltons) predicted by the X-ray and electron diffraction studies. It is likely though, as with other hydrophobic proteins, that SDS-PAGE analysis results in an underestimate (by as much as 40%, e.g., Leaver *et al.*, 1983) of the true molecular weight, bringing it into the required range. Alternatively, each subunit may be a 16k dimer (apparent molecular weight 26k by SDS-PAGE).

The connexins, on the other hand, can be removed from the junctional structure by detergent or protease treatment without loss of the normal junctional architecture suggesting they have another, non-channel role.

### *Arthropod gap junctions*

Gap junctions isolated from arthropod tissues by methods using high pH, or detergent and urea, or detergent and urea plus protease all contain a major 18k protein (Finbow *et al.*, 1984; Berdan & Gilula, 1988). Over the available common sequence (62 residues) there is 89% identity between the 18k junctional protein from the arthropod *Nephrops norvegicus* and the 16k vertebrate protein from mouse (Finbow & Findlay, in preparation). A similar high degree of evolutionary conservation has also been found in other membrane channel proteins.

As yet, connexins have not been found in arthropods (Berdan & Gilula, 1988) but it is possible that unrelated and as yet unidentified proteins fulfill equivalent roles.

The study of Berdan & Gilula (1988) showed that at early stages of isolation from crayfish hepatopancreas the gap junctions varied in thickness. The thicker forms survived treatment with high pH but only the thinner forms were present after detergent treatment. Similarly, it has been reported that disruption of mouse liver membranes at high pH produces thicker junctions than those which have been extracted with detergent and urea (Hertzberg, 1984). These different procedures could be selectively isolating different junctions but, as Berdan & Gilula (1988) point out, the thinner junctions may arise by collapse of the thicker due to loss of material (e.g., lipid and connexin-like molecules) during detergent extraction.

Confusion of gap junctions with other cellular structures seems unlikely. Despite a literature survey, Berdan and Gilula (1988) were unable to find any reports of other double membrane structures with hexagonally packed particles. Close appositions between intracellular membranes occur in some cells types (for list of examples see Berdan & Gilula, 1988), probably prior to membrane fusion events, and in thin section these double membrane regions sometimes resemble gap junctions. Freeze-fracture analysis shows, however, that these regions are particle-depleted (or particle-free) and lipid rich. These structures do not survive the detergent isolation procedures and they do not have the densely packed, often hexagonally arrayed particles seen in gap junctions. This characteristic particulate morphology provides an identifying feature which is retained in isolated

gap junctions and clearly revealed by electron microscopy of negatively stained preparations.

*The gap junctional complex*

Immunolocalisation (Traub *et al.*, 1982; Stevenson *et al.*, 1986; Zimmer *et al.*, 1987; Dermietzel *et al.*, 1987; John, 1987; Buultjens *et al.*, 1988) shows that both the 16k (or 18k) protein and the 27k (and/or 21k) connexins are intimately associated with the junctional structure and the uncoupling caused by microinjection of antibodies to these proteins into cells (Warner *et al.*, 1984; Hertzberg *et al.*, 1985; Brummer, 1988; Serras *et al.*, 1988; Willecke *et al.*, 1988) shows that both types of molecule are required to maintain active junctions. On the basis of the biochemical evidence summarized above it seems reasonable to conclude that the 16k/18k proteins are core channel components of the junctions but the role of the connexins is less clear. They may be functionally important but easily dissociated channel components, they may be associated with the junctions in some other way, or they may form a separate class of functionally similar but structurally distinct gap junctions.

The last proposal is unlikely in view of reports which show that separate injections of anti-16k, anti-21k or anti-27k antibodies into hepatocytes each reduce dye transfer to undetectable levels (Brummer, 1988; Willecke *et al.*, 1988). The 16k protein and the connexins are it seems all required simultaneously to maintain active coupling, presumably by joint participation in some form of junctional complex.

Further light is thrown on the possible role of the connexins by considering their orientation in the junctional membrane. The generally accepted model (Beyer *et al.*, 1987; Zimmer *et al.*, 1987; Figure 2) was constructed on the assumption that the connexins are channel proteins and that the protease and antibody accessible domains in isolated junctions must therefore lie on the cytoplasmic faces (Zimmer *et al.*, 1987). If this assumption is set aside in view of the data discussed above, there is no good reason for retaining the model. This allows a more logical interpretation of the cross-tissue reactivity seen in antibody uncoupling experiments.

Antibodies raised against liver 27k connexin disrupt coupling when injected into myocardial cells even though these cells synthesize a different (45k) connexin (Hertzberg *et al.*, 1985). This cross-reactivity is inconsistent with the model (Figure 2) because, as Beyer *et al.* (1987) point out, only the putative transmembrane and extracellular regions are conserved while the cytoplasmic domains, where the blocking antibodies should interact, are unique (the apparently better homology depicted in the cytoplasmic loop is due to artistic license as four insertions in the 45k connexin sequence have been collected at a single site). Without recourse to an explanation involving some form of conformational epitope, the model has to be redrawn with conserved sequences on the cytoplasmic face. This could be done by turning the model inside-out, by moving one or more of the putative trans-membrane domains into the cytoplasm or by a mixture of both.

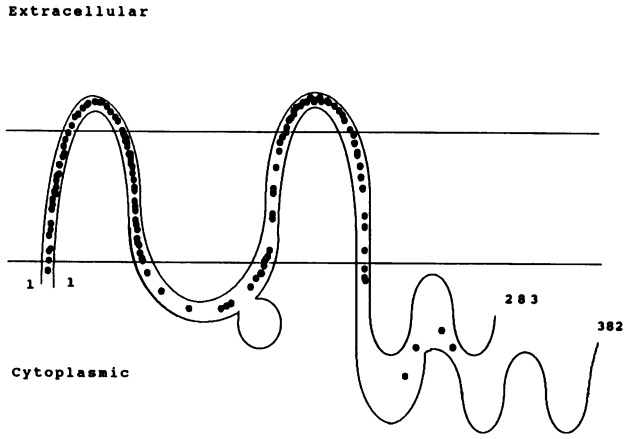


**Figure 2** Model showing the orientation in the membrane of 27k and 45k connexins. Amino acid identities are represented by the dots between the two molecules. Reproduced with permission from Beyer *et al.*, 1987.

Turning the connexin model round not only provides the necessary conserved cytoplasmic domains but also has the interesting added advantage of placing the tissue-specific C-termini on the extracellular face where they could more logically be involved in the specificity of junction formation.

The recent discovery that the *Drosophila* clock (*per*) gene affects junctional communication (Bargiello *et al.*, 1987) suggests a further component is also involved in the regulation of junctional communication. The *per* locus codes for a proteoglycan (Reddy *et al.*, 1986), a member of a complex family of extracellular matrix molecules with tissue specific distributions. Proteoglycans have also been shown to stimulate junctional coupling between primary hepatocytes in culture, possibly in a tissue specific manner (Spray *et al.*, 1987).

For these matrix molecules to play some tissue specific role in junction formation it seems reasonable to suppose that they will interact with some tissue specific component in the gap junction or its precursor formation plaque (Johnson *et al.*, 1974). The best candidates for such components are the connexins, particularly if the model is arranged, as suggested above, with the tissue specific C-terminal tail on the extracellular side. The connexins could then promote specific adhesive interactions between adjacent cells through the proteoglycans to produce the close membrane appositions required for the precursor hemi-channels to interact. The connexins may be partially or totally excluded as junctions mature but the disruption of coupling by antibodies to connexins shows they retain some essential, possibly stabilising function. If so, they should interact with some cytoskeletal component, consistent with the proposed presence in the revised model of conserved connexin domains on the cytoplasmic side of the membrane, This is also consistent with recent biochemical data which shows liver connexins co-purify with the cytoskeleton and not with the gap junctions after triton solubilization of crude plasma membrane fractions (Finbow *et al.*, 1988).

The sensitivity of the connexins to detergent extraction shows they are not tightly associated with the junctional channels and the absence of extra, non-channel particles in the freeze fracture images of gap junctions *in situ* suggests they may be organized like the transmembrane components of *zonula adhaerens*. They may fill the clear areas, sometimes arranged as particle free aisles, which are often seen in freeze-fractured junctions in different tissues (Larsen, 1977). The variable thickness of junctions isolated by different methods (see above) may be due to the presence of different amounts of connexin.

This concept of a junctional complex is more consistent with the available structural and functional data. It provides a mechanism to stabilize the coupled state, a feature which has been neglected before but which should be of particular importance in tissues like heart which suffers mechanical stress. It suggests possible mechanisms for control which are additional to channel gating, it offers an explanation for the specificity of junction formation and it provides a better basis for understanding the complex patterns of junctional communication which are formed and maintained *in vivo*.

*Specificity of junctional communication in culture*

Certain cell types in mixed cultures prefer to form homologous rather than heterologous gap junctions (Pitts & Bürk, 1976; Fentiman *et al.*, 1976). This specificity of junction formation is often accompanied by a propensity for the different cell types to 'sort out' into separate domains (Pitts, 1980). Extensive homologous coupling within the domains and infrequent heterologous coupling between cells in dif-

ferent domains results in the formation of communication compartments with well defined boundaries (Pitts & Kam, 1985). The junction mediated cytoplasmic continuity within a compartment leads to coordination of cellular activities (Pitts *et al.*, 1986). The compartmental homeostasis is of wide-ranging importance as it affects, among other things, metabolic activities (Pitts, 1971), ion fluxes (Pitts *et al.*, 1986) and cellular responses mediated by low molecular weight cytoplasmic second messengers (Kam & Pitts, 1988*a*, *b*). Junctional communication converts the individual cells within the compartments into coordinated, multicellular units and compartmentation within the overall population provides a mechanism for segregating activities at a supra-cellular or tissue level.

### *Patterns of junctional communication and communication compartments* in vivo

With the exception of a few cell types which become uncoupled during terminal differentiation, nearly all cells *in vivo* form gap junctions. However, until recently, little was known about the extensiveness of junctional communication in intact tissues or about the possible importance *in vivo* of the specificity and compartmentation seen in cultures.

The recent development of techniques which can be used to map the pathways of junctional communication in whole tissues has revealed that the extensiveness of coupling varies greatly from tissue to tissue, that complex patterns of compartmentation are formed, that cells following different pathways of differentiation are usually in different compartments, that compartmental boundaries may or may not be associated with observable morphological features and that trans-boundary coupling can be regulated in response to physiological demand (Kam *et al.*, 1986; Pitts *et al.*, 1986; Kam & Pitts, 1988*a*, *b*; Salomon *et al.*, 1988).

The pathways have been mapped in most detail in mouse skin (Kam *et al.*, 1986; Pitts *et al.*, 1986; Kam & Pitts, 1988*a*, *b*). When Lucifer Yellow (a small molecular weight fluorescent dye which passes through gap junctions but not across the non-junctional membrane) is injected into one dermal fibroblast for 5 min, it spreads detectably into as many as 2500 other dermal cells. Dye spreads through the dermal fibroblasts with no apparent limits and also into capillary endothelial cells but it does not normally spread into epidermal cells or into the epithelial cells of the sebaceous glands. The dermis appears therefore to be a continuous, very large communication compartment.

The epidermis, in contrast is divided into many small communication compartments. Dye injected into one cell for 5 min spreads only into a small column of keratinocytes made up of 5 or 6 basal cells with the over-lying cells in the differentiating spinosal and granular layers. The more differentiated, flatter cells higher up in these columns are usually arranged spirally and interdigitate with their counterparts in adjacent columns giving each dye spread a somewhat mushroon-like appearance. Small amounts of dye sometimes spread into adjacent columns (with a marked step-down in concentration at the boundary) but dye does not normally spread into the under-lying dermal cells across the more clearly defined dermal-epidermal boundary. A schematic map of the patterns of communication in mouse skin is shown in Figure 3 (similar patterns have also been observed in human skin (Salomon *et al.*, 1988).

The epidermal compartments are strikingly similar in size and organization to the epidermal proliferative units proposed on the basis of $^3$H-thymidine pulse-chase studies (Potten, 1981). This apparent relationship and the unexpected observation that epidermal hyperproliferation is associated with the breakdown of the boundary between the epidermal compartments and the underlying dermis suggests that junctional communication and communication compartmentation may play a role in proliferative control (Kam & Pitts, 1988*a*, *b*).

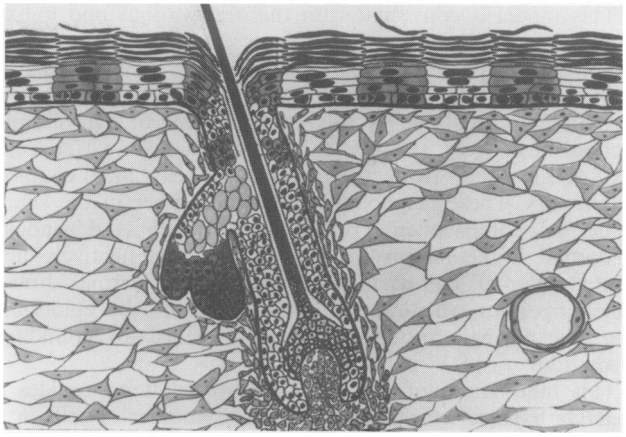

**Figure 3** Map of the patterns of junctional communication in mouse skin. The small epidermal compartments, shaded alternately in darker grey, are all equivalent. The dermal compartment shaded in lighter grey is continuous. Reproduced with permission from Pitts *et al.*, 1988.

### *Junctional communication and proliferative control*

The suggestion that junctional communication may be important in proliferative control was first put forward some years ago (Loewenstein, 1966; Furshpan & Potter, 1968; Pitts, 1971, 1978; Sheridan, 1976) and specific models have been discussed in detail by Loewenstein (1979). These ideas can be applied directly to the epidermis if small molecular weight cytoplasmic signal molecules play some mediating role in growth control, a reasonable proposition on the basis of available data. Growth factors are thought to act on a pool of committed cells in the basal layer by binding to surface receptors and stimulating the production of cytoplasmic second messengers which must reach threshold levels to initiate the processes of cell division. Second messengers (e.g., inositol phosphates, cyclic nucleotides, $Ca^{2+}$, $H^+$, $Na^+$) are small enough to pass through the gap junctional channels so the concentrations in the target cells should be reduced by diffusion through junctions into the unresponsive, supra-basal cells in the compartment. The extent of reduction will depend on the compartment size and any fall in the size, through loss from the compartment of terminally differentiated cells for example, will help to push second messenger concentrations above threshold levels, making the proliferative response to growth factors in part sensitive to epidermal thickness.

On the basis of this simple model there would, however, be a long delay between the stochastic initiation of a committed cell, once threshold levels of second messenger had been reached, and the eventual increase in compartment volume through cell growth and division. During this delay the second messenger concentrations would remain above threshold values leading to unwanted initiations of other committed cells. However, the problem can be overcome if the model is extended to take into account the observed breakdown of the dermal-epidermal boundary at times of epidermal hyperproliferation. Such breakdown occurs in epidermal tumours (Kam *et al.*, in preparation), in repeated epilation (*er*) mutant mice (Kam & Pitts, 1988*b*) which are characterized by epidermal overgrowth (Guénet *et al.*, 1979) and within 4 h of treating mouse skin with the tumour promoter TPA (12-0-tetradecanoyl phorbol-13-acetate) which induces differentiation and a burst of cell division. Initiation occurs soon after TPA treatment and the first round of mitosis starts about 24 h later. The epidermal-dermal coupling resulting from boundary breakdown will lead to loss of second messengers into the widely coupled dermis which will act as a sink to inhibit further initiations. The inhibition should last until the first cell has completed its division cycle, at which time the basal layer will reorganise itself and regenerate the dermal-epidermal boundary.

This model is consistent with the observations and unlike some earlier ideas it is not necessary to propose new, hypothetical signal molecules which use the junctional pathway. Both parts of the model are based instead on junction mediated homeostasis, in this case removal of cytoplasmic second messengers, required for proliferation, down concentration gradients from the stimulated cells into coupled neighbours. These neighbouring cells are either in the small epidermal compartments producing a thickness dependent epidermal brake on proliferation, or in the effectively unlimited dermal compartment producing a dermal brake which operates whenever the basal layer of the epidermis loses the normal, resting organization needed for boundary formation.

This modulation of growth factor stimulation provides a negative balance which is related to the number of cells in a compartment. It seems inevitable that it must operate whenever second messengers are involved in a stimulation mechanism acting on only a proportion of the cells in a coupled population. The responding cells can escape such negative control if (i) the number of non-responding cells falls, (ii) junctional permeability is decreased, (iii) the rate of production of second messenger is increased or (iv) the threshold for the response to second messenger is lowered. It should also be noted that the $K_m$ of the target protein for the second messenger will affect the threshold, offering a mechanism for the production of different sized compartments which may be important in the evolution of tissue shape.

Abnormal growth stimulation can be produced in various ways which can be understood in terms of the proposed model. For example, TPA inhibits junctional communication between certain cell types in culture (Yotti *et al.*, 1979) which fits well, but recent work has shown this effect is not observed in intact epidermis (Kam & Pitts, 1988*a*). The stimulatory effect of TPA may therefore be due to activation of protein kinase C (Castagna *et al.*, 1982) with a consequent change either in the rate of second messenger production or in the sensitivity of the response to second messengers (i.e., change of threshold). Oncogenes such as *ras* may affect second messenger production, those such as *myc* the threshold and those such as *src* junctional permeability (Atkinson *et al.*, 1981).

In *er* homozygotes, abnormal epidermal growth covers the external orifices and the pupoid-like embryos die of suffocation at birth (Guénet *et al.*, 1979). Examination of this proliferative defect in terms of the model is particularly interesting. Junctional communication is apparently normal in the mutant epidermis (consistent with grafting studies which show the lesion is not epidermal, Fisher, 1987) but is severely reduced in the dermis (Kam & Pitts, 1988*b*). This deprives the epidermis of a functional dermal brake so second messenger concentration will not be reduced after the initiation of the first committed cell in each epidermal compartment. This should result in the stimulation of too many cells at each round of replacement and also explain the observed, extensive breakdown of the dermal-epidermal compartment boundary (Kam & Pitts, 1988*b*).

### *Compartmentation and the emergence of difference during organogenesis*

Direct intercellular communication via gap junctions in animals, or via the functionally similar but structurally distinct plasmodesmata in plants, appears to be a general feature of complex multicellular organization. The partial syncytial state allows coordination through shared pools of small ions and molecules while allowing cells to retain distinctive activities through their macromolecules. However, because small ions and molecules are involved in so many cellular processes, there is restraint on the expression of differences within coupled populations. It seems that major differences, as occur in the production of different tissue types during development, require better isolation in the form of compartmentation. The compartment boundaries can therefore play a controlling role in first allowing and then protecting these differences. The breakdown of the epidermal-dermal boundary under particular conditions and its proposed role in proliferative control may be just one example of a more widely used mechanism to regulate the emergence of differences between different parts of a developing organism. Understanding junctional communication and the mechanisms of specificity at a molecular level should provide appropriate tools for experimental analysis of these ideas.

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