COMMENTARY

The gap junction

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Introduction

Gap junctions are intercellular structures that link cells and allow them to exchange ions and small metabolites without recourse to the extracellular space (for reviews and early references, see Goodenough, 1979; Loewenstein, 1981). They are found in every organ including the nervous system, where they form electrical synapses; the only exception being fully differentiated skeletal muscle. Gap-junctional communication is present between all cells in early embryos, regardless of eventual developmental fate, at times when cellular interactions are known to be taking place (Potter *et al.* 1966). The widespread occurrence of gap junctions has led to the proposal that they play an important part in coordinating cellular signals, including those involved in growth control and embryogenesis.

The aim of this review is to outline current opinion on some properties of gap junctions. It has not been possible to be comprehensive. I have not considered work relating to gap junctions in the lens of the eye (see Kistler *et al.* 1985) or gap junctions in arthropod species (except where they illuminate developmental issues) and have focussed on areas where controversy exists, or where there have been substantial recent advances in understanding. A useful review has been published by Spray & Bennett (1985).

Ultrastructure

Early ultrastructural evidence suggested that the gap junction channel (or connexon) is composed of six protein subunits surrounding a central core; this view still holds. The central core is supposed to reflect the conducting channel, although direct evidence for the location of the cell-to-cell channel is still lacking. The gap junction is a highly ordered, stable structure that permits application of X-ray diffraction techniques (Makowski *et al.* 1977), which has led to the suggestion that the protein may exist in the B-helical form. Unwin & Zamphighi (1980), using electron diffraction, proposed that the connexons in each cell membrane abut end on, with central cores apposed, but with a slight tilt, and that channel closure may be achieved by twisting each unit, so reducing the width of the central core. The appearance of isolated gap junctions, viewed *en face* with negative staining, changes little after treatment with enzymes that progressively digest the constituent protein (Zimmer *et al.* 1987). Even when, according to gel electrophoresis, the major protein isolated from such junctions had broken down to 4–6K ($K = 10^3 M_r$) fragments, the appearance in the electron microscope was virtually indistinguishable from undigested junctions. Thus gross ultrastructural appearance may be a poor guide to the integrity of the junction, suggesting that the characteristic ultrastructural features arise from a rather small, membrane-spanning 'core' of protein.

The identity of the gap junction protein

Until recently, the view that gap junctions are largely constituted from a single protein dominated the field, with a 26–28K protein as the major contender. An important advance has been the isolation of cDNA clones coding for the '27K' protein; sequences derived from clones from rat liver (Paul, 1986) and human liver (Kumar & Gilula, 1986) are almost identical. The cDNA predicts a protein molecular weight of 32K. Previous estimates of the size of the major gap junction protein ranged from 10K up to 66–68K. A number of factors have combined to generate this diversity. (1) The 27K protein is sensitive to proteolysis, accounting for the lower range of apparent molecular weights. (2) It tends to form both dimeric and trimeric forms, accounting for the larger values. (3) It behaves anomalously on polyacrylamide gels (Green *et al.* 1987) and, depending on gel percentage, the monomer can run at either 27 or 32K, while the dimer can be at 47, 54 or 64K. It therefore now seems most likely that the molecular weight of this protein is as predicted from the cDNA clone, i.e. 32K, with a dimer of 64K. Apparent molecular weights between 27 and 64K represent monomers and dimers. For the remainder of

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this article this protein will be called the 32K gap junction protein. Models and hydrophobicity plots (see Zimmer *et al.* 1987; Paul, 1986) suggest that this protein crosses the membrane four times and that both amino and carboxy terminals lie on the cytoplasmic side.

But this is not the end of the story. Evidence is accumulating to suggest that the gap junction protein may not be the same in all tissues, and that junctions in a single tissue may contain more than one protein. Thus a 45K protein has been extracted from gap junctions in heart muscle (Majunath *et al.* 1984). This is not a dimeric form of the liver protein, since sequencing of the amino terminus suggests that there is about 50% homology between liver and heart proteins (Nicholson *et al.* 1981). Furthermore, a 21K protein, which also shares some homology with the 32K protein at the amino terminus and can co-exist in a single junction with the 32K protein, has been identified (Look *et al.* 1987; Nicholson *et al.* 1987; Revel *et al.* 1987). Finally, it has been claimed that the major constituent of gap junctions is a completely different protein with an apparent molecular weight of 16–18K, which appears to have no sequence homology or immunological cross-reactivity with the 32K or 21K proteins (see Pitts & Finbow, 1986). The 16–18K candidate displays very different properties from the 32K protein. It is resistant to proteolysis and apparently shares some immunological determinants with a protein extracted from arthropod gap junctions.

An important question is whether there are proteins associated with the gap junction that are not constituent proteins, but are involved in functional control. Such proteins might or might not be isolated along with gap junctions. Calmodulin has been suggested as one such protein (Kumar & Gilula, 1986; Zimmer *et al.* 1987; Van Eldik *et al.* 1985) and some proteins isolated from gap junction preparations could subserve such a role. It is entirely possible that some of the candidates for 'gap junctions' proteins fall into this class.

Immunological recognition of gap junctions and gap junction protein

A variety of methods have been used to raise antibodies against the gap junction and its constituent proteins. Isolated junctional plaques (Traub *et al.* 1982; Hertzberg & Skibbens, 1984; Paul, 1986) or electrophoretically eluted protein (Traub *et al.* 1982; Warner *et al.* 1984) have been used as immunogen and the antibodies affinity-purified against junctional plaques (Dermetziel *et al.* 1984) or eluted protein (Hertzberg & Skibbens, 1984; Warner *et al.* 1984; Paul, 1986). All these antibodies recognize proteins isolated from gap junctions on immunoblots. Antibodies against peptides from the amino-terminal sequence of the 32K protein also recognize the 32K protein on immunoblots (Zervos *et al.* 1985). Monoclonal antibodies (Dermetziel *et al.* 1987; Stevenson *et al.* 1986) that recognize the 32K protein are now available. Antibodies that recognize the 16–18K protein (Pitts & Finbow, 1986) have also been described.

Such diverse sources of immunogen and affinity-purification procedures generate antibodies against a variety of antigenic sites on gap junction proteins and it is not surprising that the specificity of the available antibodies varies. Some recognize protein isolated from gap junctions of both vertebrate and invertebrate species other than the arthropods (e.g. see Warner *et al.* 1984; Fraser *et al.* 1987; Lee *et al.* 1987), while others recognize protein isolated from gap junctions of most vertebrates (e.g. see Hertzberg & Skibbens, 1984). Tissue specificity within species has also been noted (e.g. see Dermetziel *et al.* 1984). This variability presumably reflects both conservation and divergence of antigenic sites between junctions of different origin. The monoclonal antibodies available to date are limited in their cross-reactivity, as found for many monoclonals against membrane proteins.

The availability of antibodies should advance identification of candidate gap junction proteins that are located at gap junctions, rather than other structures in the cell and plasma membranes. Such evidence, using immunofluorescence and immunogold labelling, is now available for the 32K and 21K candidate proteins (Dermetziel *et al.* 1987; Look *et al.* 1987; Paul, 1986; Young *et al.* 1987; Stevenson *et al.* 1986), adding strength to the view that these two proteins are associated directly with gap junctions. The antibody to an amino-terminal peptide of the 32K protein does not label intact junctions (Zimmer *et al.* 1987); presumably the binding site is masked. However, evidence that the 16–18K candidate (Pitts & Finbow, 1986) is associated specifically with gap junction structures is still lacking, although decoration of isolated plaques that lack adjacent plasma membranes, viewed *en face* with negative stain, has been reported.

No antibodies recognizing portions of protein that lie in the 'gap' or are exposed on the external face of the membrane before junction formation occurs have yet been reported.

Functional studies

Physiological properties

The range of treatments that influence functional communication through gap junctions is considerable. When the free intracellular concentration of calcium ions rises, gap junctions close (Rose & Loewenstein, 1976), and one functional role of calcium ions is to

ensure that gap junctions in damaged cells shut to prevent loss of ions and metabolites from neighbouring cells. Reversible effects of calcium, at levels that do not cause complete closure, have been reported (Noma & Tsuboi, 1987). Gap junctions are sensitive to intracellular pH (Turin & Warner, 1977, 1980; Spray *et al.* 1981*a*), with junctional conductance falling as the hydrogen ion concentration rises. The block brought about by a fall in pH is completely, and rapidly, reversible even after complete closure of junctional channels. The effective pH range varies from system to system. Amphibian embryonic cells are most sensitive in the region of pH 7·0 (Turin & Warner, 1980; Spray *et al.* 1981*a*), while gap junctions in the liver and heart respond only when the pH falls below pH 6·5 (Spray *et al.* 1982; Noma & Tsuboi, 1987). The calcium sensitivity of gap-junctional conductance varies also. Gap junctions in heart muscle begin to close at free calcium concentrations within the physiological range (pCa 7–6; Noma & Tsuboi, 1987), while gap junctions between embryonic cells are much less sensitive (Spray *et al.* 1982). Calcium ions and hydrogen ions can act independently to reduce junctional conductance (Spray *et al.* 1982; Noma & Tsuboi, 1987).

In heart muscle (Noma & Tsuboi, 1987) the relation between pH and junctional conductance is not affected by variations in calcium concentration. However, the sensitivity of the junction to calcium ions depends on the ambient pH: as pH falls, so the junction becomes less sensitive to a rise in calcium. This effect of hydrogen ions operates above pH 6·5 and suggests that when hydrogen binds to the calcium binding site it cannot reduce junctional conductance. If this is true, then in heart muscle there ought to be an increase in junctional conductance between pH 7·4 and 6·5 as calcium is displaced from its binding sites. This effect is most marked at calcium levels achieved during tension development (pCa approx. 6·0), when a pH fall of 0·5 unit is sufficient to counteract completely the effect of calcium on junctional conductance. It follows that any fall in pH during electrical or contractile activity will reduce the sensitivity of the gap junction to alterations in calcium concentration. This may help to explain the apparently paradoxical observation that cardiac cells do not uncouple transiently with each beat, despite the increase in intracellular calcium necessary to sustain twitch tension.

Some gap junctions are sensitive to voltage. The classical example is the crayfish asymmetric giant synapse, which rectifies when a voltage is applied across the junction in such a way that an action potential is transmitted in one direction only. Symmetrical voltage dependence has been observed for gap junctions between blastomeres of the amphibian and *Fundulus* embryos (Spray *et al.* 1981*b*). Dependence of junctional conductance on the absolute resting potential, rather than the potential imposed across the junction, has also been reported (Obaid *et al.* 1983).

One of the most interesting advances has been the recognition that hormones can modulate gap-junctional communication. In the uterus smooth muscle cells are weakly interconnected through gap junctions. However, prior to parturition, and in response to circulating hormones, there is massive synthesis of gap junctions, probably induced by an increase in intracellular levels of the second messenger, cyclic AMP, so that uterine contractions can be synchronized during birth. Once the foetus is expelled these gap junctions are rapidly internalized and the uterine muscles return to the preparturition state, with few intercellular gap junctions (Garfield *et al.* 1977). The ability of cyclic AMP to upregulate junctional communication extends to other cell types and other hormones that induce a rise in intracellular cyclic AMP (e.g. see Radu *et al.* 1982). Conversely, dopamine has been shown to reduce gap-junctional communication between retinal horizontal cells (Neyton *et al.* 1985).

An interesting extension has come from studies in which junctional communication was examined in a temperature-sensitive mutant of cells infected with Rous sarcoma virus at permissive and non-permissive temperatures. When the *src* gene is active, communication through gap junctions is greatly reduced (Azarnia & Loewenstein, 1984). Cells transformed with simian virus 40 also show defective communication through gap junctions (Atkinson *et al.* 1981; Azarnia & Loewenstein, 1984). Such experiments reinforce suggestions that junctional communication may be involved in growth control, although defective junctional communication is probably not causally linked to the tumorogenicity of cancerous cells. The ability to establish communication between normal and cancerous cells may be more important.

Such functional diversity demands variety in the regulation of gap junction properties and supports the proposition that more than one protein may be present, either within or associated with gap junction structures.

Two studies on the permeability of gap junctions deserve comment. Lawrence *et al.* (1978), in an elegant study using co-cultures of heart and ovarian granulosa cells, demonstrated that second-messenger molecules generated by the action of noradrenaline on heart cells (evidenced by increased frequency of beating) and of follicle-stimulating hormone (FSH) on granulosa cells (evidenced by the secretion of plasminogen activator) could pass through gap junctions. When heart cells were in contact with granulosa cells, application of noradrenaline provoked granulosa cells to secrete plasminogen activator, while FSH increased the beat frequency of the cardiac cells. The transferred second messenger is probably cyclic AMP. More recently it

has been shown that photoreceptor cells in the hydroid *Lobelia* require stimulation through gap junctions with their support cells to induce light generation (Dunlap *et al.* 1987).

The conductance generated by a single connexon within the gap junction is difficult to derive from macroscopic currents, because the number of connexons within a single gap junction is hard to estimate and the currents are large. Single channel events have now been detected in pairs of cells, where the problems imposed by the high conductance of gap junctions were avoided by examining junctions during closing (Neyton & Trautman, 1985) or formation (Veenstra & De Haan, 1986), or by incorporating the 32K gap junction protein into lipid bilayers (Young *et al.* 1987). Such experiments give an average single channel conductance of 160–300 pS, although the observed range is large (50–1000 pS). These measurements bear upon a still unresolved problem: whether gap junction channels can exist only in the fully open or fully closed condition or whether there are substates that might correspond to junctional channels in the partially open (or partially closed) position. This issue is important because a number of studies suggest that ionic coupling through gap junctions may be possible when the transfer of fluorescent, larger molecules is restricted. Such restriction could arise because only a small number of channels are fully open so that the time taken for sufficient dye to move through junctional channels into adjacent cells and be detected would be very long. Alternatively, junctional channels may exist in the partially open state. As the techniques of determining single-channel conductance in gap junctions improve, an answer to this important question should become available.

Gap junction antibodies

The identification of the sites that control permeation through gap junctions can, in principle, be explored by testing whether antibodies raised against gap junction protein interfere with junctional conductance. Antibodies raised and purified against the 32K protein, when injected into cells, inhibit both dye transfer and electrical coupling in the *Xenopus* embryo (Warner *et al.* 1984), in *Hydra* (Fraser *et al.* 1987) and in the eight-cell mouse embryo (Lee *et al.* 1987). In the amphibian embryo, monovalent Fab fragments prepared from the antibodies also blocked junctional communication, indicating that the block did not simply arise from a screening effect of the immunoglobulin G molecules (IgGs). A block of junctional communication was also achieved by intracellular injection using the antibody described by Hertzberg & Skibbens (1984) in cultured heart cells, liver cells and sympathetic neurones (Hertzberg *et al.* 1985). In all these cases, IgGs from pre-immune serum were without effect. The results imply that antigenic determinants on the cytoplasmic face of the junction are recognized by the antibodies. To date there are no published reports testing other gap junction antibodies for the ability to achieve functional block.

The fact that antibodies raised against gap junction protein(s) or gap junctions can prevent functional communication constitutes an important part of the evidence that the antigen is truly related to gap junctions. However, there are important caveats that should be applied to such demonstrations. Antibodies might have other intracellular effects that lead to a rise in calcium or hydrogen ion concentration. The possibility of bulk screening of the junctional channel by large IgGs binding to sites near the functional site has not been eliminated and may prove a problem even with peptide antibodies. It is therefore important to show that monovalent Fab fragments block junctional communication also (see Warner *et al.* 1984). The demonstration that antibodies directed against some sites on gap junction protein(s) are unable to block junctional communication would provide an important counter-argument to non-specific screening by large IgGs bound to sites other than those controlling junction permeation. The difficulty of obtaining pure immunogen, of known origin, means that antibodies (both monoclonal and polyclonal) used for functional studies may be directed against components other than constituent proteins (accessory proteins, for example). This may arise from minor, highly antigenic, components in protein preparations, although such complications are more likely when whole gap junction preparations are used as the immunogenic stimulus. The ability of an antibody to decorate junctional structures is not firm proof against this possibility, since any accessory protein might be attached to the junction itself. Whether such problems will prove to have complicated results obtained to date remains to be seen. Anti-peptide antibodies may help to resolve some of these issues. Nevertheless, despite these caveats, the ability of an antibody to inhibit junctional communication must remain an important part of evidence to show that a particular protein is gap-junction-related.

A role for gap junctions during development?

A functional role for gap junctions during development has become increasingly likely. Evidence that groups of cells lose the ability to communicate with each other as they diverge along different developmental pathways has been obtained in a variety of systems (for review, see Wolpert, 1978). More recently it has been shown that junctional communication between the epithelial cells of larval insects varies according to cell location. Thus, cells that lie within the same segment freely exchange small molecules such as Lucifer Yellow.

However, cells that lie on either side of the segment border show good ionic coupling, while the transfer of Lucifer Yellow is highly restricted (Warner & Lawrence, 1973, 1982; Blennerhassett & Caveney, 1984), despite the presence of equivalent numbers of intercellular gap junctions both within segments and at the segment border (Lawrence & Green, 1975). These are important observations because the segment border marks the site of a lineage compartment (Lawrence, 1973) and form a developmental boundary (Locke, 1959), where cell–cell exchange of putative morphogens might be expected to be restricted. Weir & Lo (1984) have reported that similar restriction patterns for dye transfer exist also in the *Drosophila* imaginal wing disc, although another study (Fraser & Bryant, 1985) did not confirm these observations. Patterns of dye transfer that vary with cell position have been reported in the early *Xenopus* embryo (Guthrie, 1984); at the 32-cell stage, cells lying in future dorsal regions of the animal pole communicate with their neighbours both more frequently and more extensively than those in future ventral regions.

Whether these patterns of dye transfer are linked directly to a developmental role for gap junctions is unknown. But undoubtedly the strongest evidence for a functional role for gap junctions in development is emerging from experiments using antibodies to block junctional communication. Thus in the *Xenopus* embryo, blocking junctional communication with an antibody (Warner *et al.* 1984), which maintains the inhibition at least up to the early neurula stage (Warner & Gurdon, 1987), produces pronounced patterning defects in the region derived from the antibody-injected cell. In *Hydra* antibody-induced block of junctional communication interferes with head patterning (Fraser *et al.* 1987). In the mouse, blocking junctional communication leads to decompaction of the communication-incompetent cells (Lee *et al.* 1987).

Gap junction antibodies can help also to define those developmental interactions that do *not* take place through gap junctions. Thus the induction by vegetal pole cells of muscle gene activation in mesoderm cells of *Xenopus* proceeds unimpaired, even when communication through gap junctions is completely inhibited (Warner & Gurdon, 1987), implying that this particular aspect of meoderm induction does not require gap junctions.

The demonstration that some interactions apparently proceed normally, despite a block of junctional communication, constitutes substantial evidence that antibody-mediated blocks of gap junctions do not have their developmental effects simply because they generate toxic or entirely non-specific effects. However, they do not prove it conclusively. The only way that definitive proof will be provided is by more extensive experimentation on a variety of systems, using a range of antibodies known to inhibit functional communication.

Recent experiments in the field of gap-junctional communication have provided some remarkable clarifications (physiological control and in development) and generated some hotly debated controversies (one gap junction protein, which and/or how many?). The prospects offered by molecular cloning and site-specific antibodies should ensure that this area continues to generate excitement.

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