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COMMENTARY

Is the gap junction channel - *the connexon* - made of connexin or ductin?

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"For a' that, an a' that
Our toils obscure an a' that"
Robert Burns

INTRODUCTION

The liver gap junction and *Halobacterium* purple membrane were among the first organised membrane complexes to be isolated. The retention of their paracrystalline form during isolation provided opportunities for physical analysis and allowed early glimpses of the structural organisation of integral membrane proteins. The seminal study of Henderson and Unwin (1975) on the purple membrane, using electron diffraction analysis and image reconstruction, revealed a detailed topology of the subunits that could be related to the structure of the component protein, bacteriorhodopsin. The work provided a framework for understanding the helical nature of the protein within the membrane bilayer and this model now serves as a structural archetype for a superfamily of proteins with seven transmembrane α-helices. Other integral membrane proteins have now been crystallised that confirm the suggestion from Henderson and Unwin's study that the secondary structure and packing features of such proteins are very similar to those of their water-soluble counterparts.

Not long after Henderson and Unwin's paper, a model was produced for the gap junction channel - *the connexon* - based on the X-ray diffraction and EM studies of Caspar, Makowski and Goodenough (Caspar et al., 1977; Makowski et al., 1977). The resolution was lower (20 Å compared to 7 Å for the purple membrane), but the general characteristics of the membrane-spanning channel subunits could be discerned. The main features of this model have been confirmed by further X-ray diffraction analyses (Makowski, 1988; Tibbits et al., 1990) and other imaging techniques including electron and cryo-electron microscopy (Unwin and Zampighi, 1980; Unwin and Ennis, 1984), and atomic force microscopy (Hoh et al., 1991). As yet, however, there has been little improvement in resolution and, unlike the purple membrane and bacteriorhodopsin, it has not proved possible satisfactorily to relate the image of the connexon to the structure of its putative component protein, *connexin*.

Connexins are a family of proteins ranging in size from 26 kDa to 50 kDa and are expressed in a cell-specific manner. These proteins are generally held to be the sole components of the connexon. There are, however, some fundamental observations that challenge this dogma.

Firstly, gap junctions are universal features of metazoan animals but, despite many attempts, no connexins or related proteins have been found in arthropods or other invertebrates (although a distant relative has been found in plants; Meiners et al., 1991). This is in spite of the successful isolation of gap junctions from a variety of arthopod tissues (Green et al., 1983; Finbow et al., 1984; Berdan and Gilula, 1988; Ryerse, 1989) and recent imaging studies which show that the connexon architecture of these junctions is very similar to that of their vertebrate counterparts (Sikerwar et al., 1991; Holzenburg et al., 1993). Secondly, connexins can be removed during isolation of gap junctions from rodent liver. The connexin-free structures produced in this way (Finbow et al., 1983; Finbow and Meagher, 1992) have the characteristic features of gap junctions (double membranes containing hexagonally arrayed particles) and similar dimensions (thickness, particle size) to the structures imaged by Caspar et al., and Unwin et al. (Caspar et al., 1977; Makowski et al., 1977; Makowski, 1988; Unwin and Zampighi, 1980; Unwin and Ennis, 1984). Connexin-free gap junctions have likewise been isolated from bovine brain. These structures also contain typical paracrystalline arrays of connexon-like particles and have central deposits of negative stain within them, indicative of the existence of central pores (Dermietzel et al., 1989).

These observations lead to the conclusion (Finbow et al., 1983; Berdan and Gilula, 1988; Dermietzel et al., 1989; Finbow and Meagher, 1992) that there are forms of gap junction that are not made of connexins. The presence of such gap junctions in preparations made from rodent liver raises the worrying possibility that the imaging studies have been based on structures that do not contain connexins. This suggestion flies in the face of current dogma but it has an important attraction in that it would provide a solution to the long-standing problem (see below and Milks et al., 1988; Makowski, 1988; Tibbits et al., 1990; Sosinsky, 1992) of a model for the connexon that cannot accommodate the large cytoplasmic domain of connexin. It would also allow a revised consideration of the structure and function of the connexins, a complex and potentially very interesting family of proteins, unshackled by the assumption that they must be fitted into the current model of a gap junction channel.

Key words: gap junction, connexon, connexin, ductin, V-ATPase

The protein component of connexin-free liver gap junctions and of arthropod gap junctions was identified (Finbow et al., 1983, 1984) not long after the discovery of the first connexin. This protein, recently called *ductin* (Holzenburg et al., 1993), has a molecular mass of 16-18 kDa. It is very hydrophobic (soluble in chloroform-methanol) and has a propensity to aggregate in SDS buffers, particularly after heating. It has been found in gap junction preparations isolated from many different sources, including various mammalian tissues and cell lines (Finbow et al., 1983; Buultjens et al., 1988), chicken and *Xenopus* liver (Buultjens et al., 1988), crustacean hepatopancreas *(Nephrops norvegicus*, Finbow et al., 1984; *Homarus americanus*, Holzenburg et al., 1993; crayfish, Finbow et al., 1984), insect midgut *(Manduca sexta*, M. E. Finbow, N. J. Lane and J. B. C. Findlay, unpublished results) and squid digestive gland (Finbow et al., 1988).

Apart from its presence in gap junction preparations there are several other lines of evidence showing that ductin is a component of gap junctions and involved in intercellular communication. Antibodies to ductin bind to isolated gap junctions (Buultjens et al., 1988), gap junctions in plasma membrane-enriched fractions (Leitch and Finbow, 1990), and gap junctional regions in tissue sections (Leitch and Finbow, 1990). In *Drosophila*, immuno-staining (Bohrmann, 1993) produces spots in regions of contact between cells that are smaller but otherwise similar to the those formed in vertebrate tissue sections by antibodies to connexins. The anti-ductin antibodies also inhibit junctional communication when injected into mammalian (Finbow et al., 1993), molluscan (Serras et al., 1988) or insect cells (Bohrmann, 1993). And reconstitution into lipid bilayers produces channels permeable to hydrogen ions (Sun et al., 1987) and small molecules (Israel et al., 1986; Birman et al., 1990).

These data, together with the similar evidence produced for connexins (for review see Dermietzel et al., 1990), show that both types of molecule are involved in gap-junctional communication but provide only equivocal evidence for the identity of the channel protein. For example, the antibody microinjection studies do not distinguish between those molecules involved in producing or controlling the functional structure and those molecules that make the channels. This also holds for expression studies (Swenson et al., 1989; Eghbali et al., 1990) where increasing levels of connexins can stimulate junctional communication. In this context it is worth noting that expression of cadherins can also increase junctional communication (Mege et al., 1988) but it is not suggested that these molecules form cell-cell channels.

EM immunocytochemistry provides a more direct test for structural components but gap junctions in situ, or in membrane fractions, may be more complex in composition than the purified gap junctions used to generate the presently accepted models that contain only channels and phospholipid (Makowski et al., 1977; Unwin and Zampighi, 1980 and see below). The amount of material lost during isolation depends on the extraction procedure and may account for reported variations in junctional width (e.g. gap junctions extracted with only high pH treatment are ~20% thicker than those extracted with Triton X-100 and *N*-lauroylsarcosine; Finbow and Meagher, 1992).

Interpretation of the reconstitution studies depends on sample purity, particularly when channel formation is assessed with the single-channel recording techniques. In principle, a signal could be generated by the assembly of only six molecules (out of the 10^{12} or so added).

With these difficulties in mind we have turned to structural considerations to help resolve the problem of the identity of the channel protein. The primary structures of the candidate proteins are known and there is good agreement, from a variety of experimental approaches, on the structure and dimensions of the channel. In the remainder of this Commentary we examine the evidence for the currently accepted model of the connexon and then assess the compatibility of the model with the structures of the connexins and of ductin. We conclude that ductin is the more likely component.

STRUCTURE OF THE CONNEXON

The accepted model of the connexon (see Fig. 1) has been produced by a variety of imaging techniques including conventional thin-section (Caspar et al., 1977), negative stain (Caspar et al., 1977; Unwin and Zampighi, 1980; Baker et al., 1985; Sosinsky et al., 1990), low-dose cryo-electron microscopy (Unwin and Ennis, 1984), X-ray diffraction (Makowski et al., 1977, 1984; Makowski, 1988; Tibbits et al., 1990) and atomic force microscopy (Hoh et al., 1991). They all reveal much the same structure, showing the connexon to be a cylinder of protein 70-75 Å in length and 60-65 Å in diameter (Fig. 1a) with an axial water-filled channel 15-20 Å in diameter (Fig. 1d). Each connexon joins end-to-end with a connexon in the apposing membrane of another cell to provide a direct aqueous pathway between the cytoplasms of the coupled cells. Each cylinder is formed from six similar-sized subunits arranged symmetrically around the central channel (Fig. 1d). The cylinders may taper a little at the extracellular face (Unwin and Ennis, 1984; Fig. 1a), but the cross-sectional area of each subunit in the hydrophobic core of the bilayer is the required size to accomodate four α-helices (Unwin, 1986; Makowski, 1988; Milks et al., 1988; Tibbits et al., 1990).

Recent data (Tibbits et al., 1990), based on X-ray diffraction studies, suggest that about 60% of the component protein is α-helical. The diffraction pattern can be best fitted by arranging the protein in the membrane as a hexamer of four α-helical bundles each with an average helix length of 35 Å and located at a mean radius of 24 Å from the centre of the channel (Tibbits et al., 1990).

These data provide a basis for estimating subunit mass. The average length of each of the four α-helices in the transmembrane region is equivalent to 25 residues (Tibbits et al., 1990), making a total of 100 residues per subunit. This value, coupled with the estimate that 60% of the component protein is located in the α-helices, generates a subunit size of 165 residues (~18,000 Da of protein). Connexon mass can also be estimated from volume calculations but these are unduly influenced (to the power of 2) by estimates of connexon diameter. The precise location of the connexon

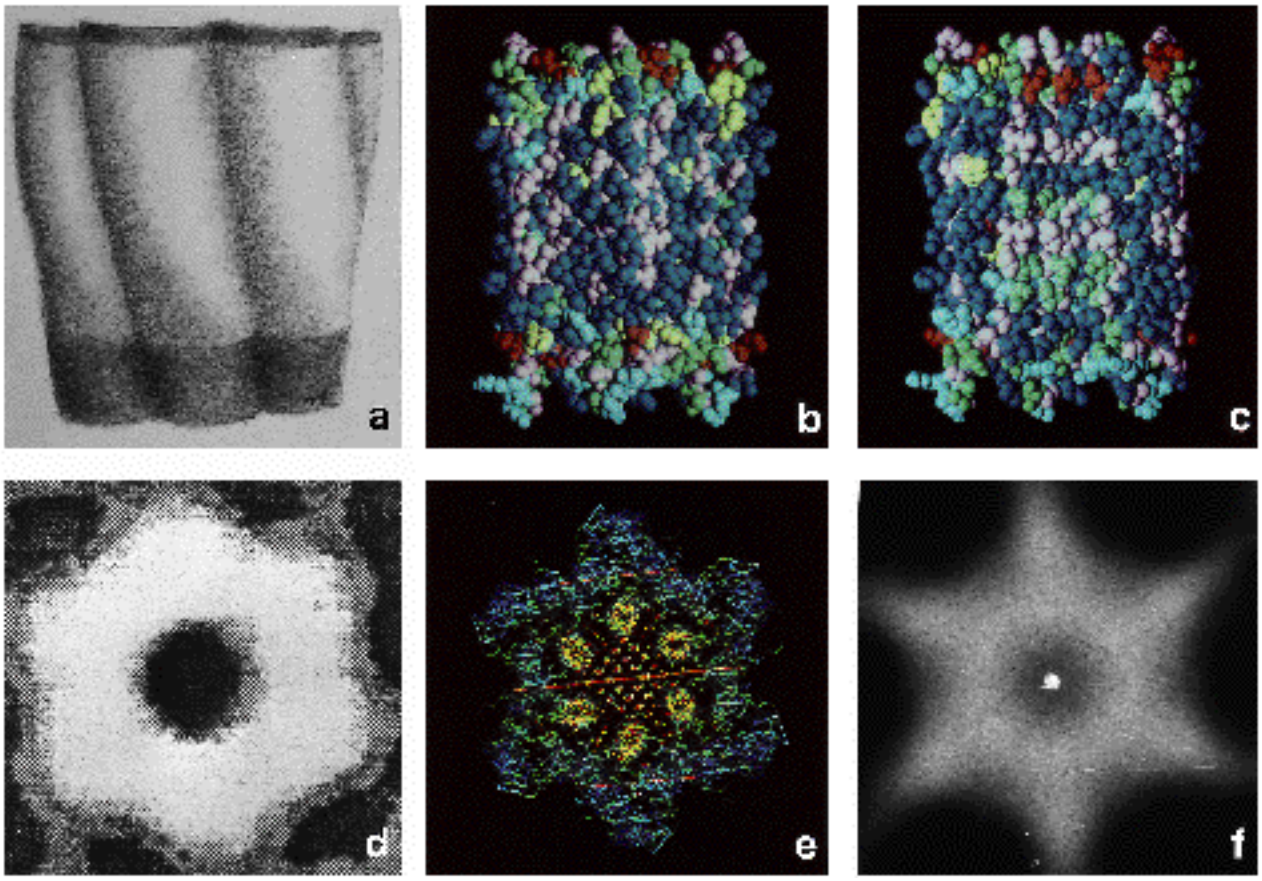

Fig. 1. Longitudinal and transverse views of images of the connexon channel and of the model of the ductin hexamer. (a) The connexon channel image was re-drawn from the model of Unwin and Ennis (1984). The lighter region shows the transmembrane segment. The cytoplasmic surface is uppermost. (b) and (c) Space-filled model (b) and longtitudinal section (c) of the ductin hexamer (taken from Finbow et al., 1992). The extent of the region of membrane contact can clearly be seen in (b) as a broad band of hydrophobic (dark blue) and neutral (magenta) residues In (c) the hexamer has been split in half to show the putative channel-lining residues. As in (a) the cytoplasmic surface is uppermost. Colouring scheme: hydrophobic (Val, Leu, Ile, Met, Cys and Phe) in dark blue; Tyr in yellow; negatively charged (Asp and Glu) in red; positively charged (Lys, Arg and His) in light blue; neutral (Gly, Ala, Pro) in magenta; polar (Ser, Thr, Gln and Asn) in green. (d) Fourier averaged model image of a negatively stained (uranyl acetate) mouse liver connexon from Baker et al., (1985). The image shows a transverse view of the connexon and the stain-filled area in the centre delineates the axial channel. (Reproduced with permission from the Journal of Molecular Biology.) (e) Transverse view of the model of the ductin hexamer complex (cf. b and c) shown with water molecules (yellow and red) in the central channel. The helices of each ductin polypeptide are arranged in a diamond shape with the first putative transmembrane helix (in yellow) of each polypeptide facing the channel. In this model the helices are arranged anti-parallel in clockwise rotation. (f) Reconstructed projection of the unit cell connexon of *Nephrops* gap junctions after optical filtering and rotational averaging. The junctions contain ductin as the principle protein component and are negatively stained with uranyl acetate as in (d). The darker, stain-filled area in the centre delineates the axial channel. See Holzenburg et al. (1993) for further details. All six images are shown with similar magnifications. The longer orange bar in (e) is 40 Å.

boundary is difficult to determine but a recent estimate generates a maximum subunit volume equivalent to 24,000 Da of protein (Makowski, 1988). Mistakes in the assumptions that lead to this value could produces errors of 10-20% but, according to Makowski (1988), they cannot explain the difference between the calculated mass and the actual mass of the liver connexin (32,000 Da).

These studies have been confined to gap junctions isolated from rodent liver but recent structural studies (Yeager and Gilula, 1992), based on analyses of thin-sectioned and negatively stained preparations of gap junctions from heart, show that connexon dimensions and architecture in this tissue are very similar to those of the liver connexon. Likewise, an image reconstruction study on images of negatively stained gap junctions isolated from the hepatopancreas of the lobster, *Homarus americanus*, has revealed a remarkably similar picture of the constituent connexon (Sikerwar et al., 1991). It seems reasonable to conclude therefore, that connexon structure in gap junctions isolated from different tissues and phlya is highly conserved.

STRUCTURE OF CONNEXIN

The sequences of about ten different connexins (Cx) have been determined, mostly from analyses of genomic or cDNA (see Bruzzone et al., 1993, for references). They vary in size from 26 kDa to 50 kDa and are expressed in a cell-

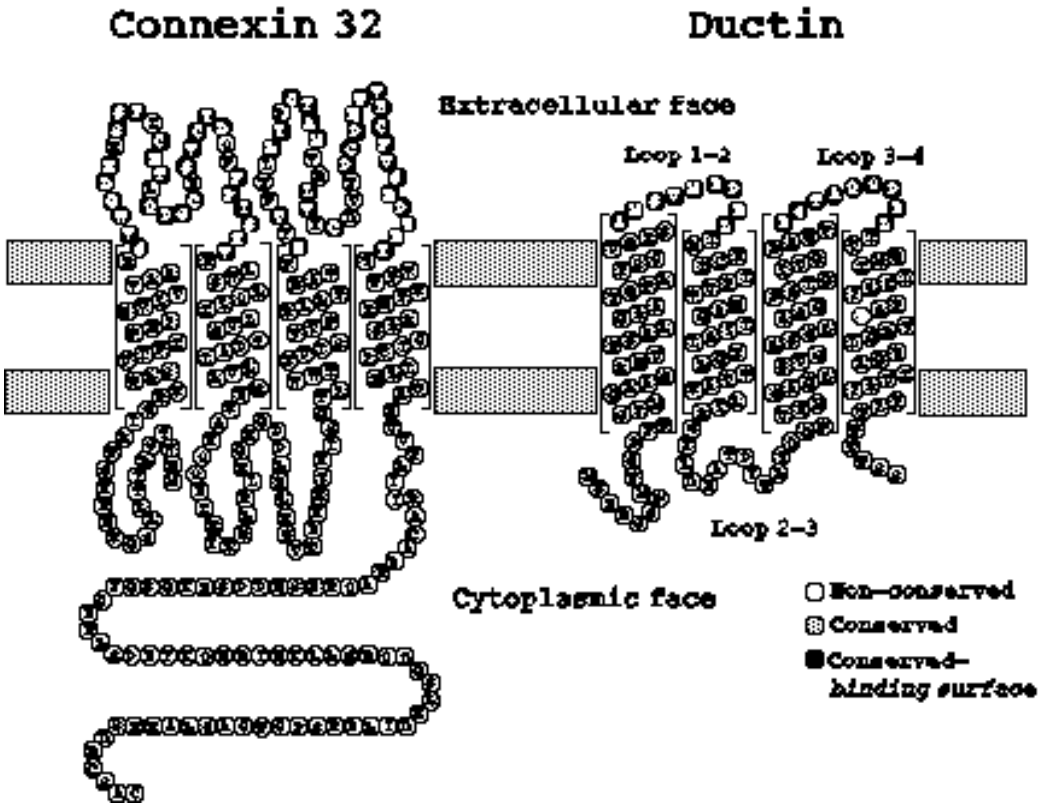

Fig. 2. Sequences and proposed dispositions of Cx32 and ductin. The disposition of Cx32 is taken from Milks et al. (1988). Residues identical or similar to those in Cx43 have been marked in grey for regions of the polypeptide thought to lie within the membrane or cytoplasmic surface, or black for the extracellular region thought to be the site of connexon attachment. Cx43 is believed to extend a further 80 residues on the cytoplasmic surface. The ductin sequence has been arranged as a tandem repeat aligning on the extracellular proline residues in loop 1-2 and loop 3-4 (see Finbow et al., 1992; Holzenburg et al., 1993). Conserved residues (from yeast to plant and animal forms) have been marked in grey or black. The DCCD reactive glutamic acid residue in the centre of the fourth transmembrane segment is marked with white lettering.

specific manner. They all show homology over the N-terminal 20 kDa or so, but the length and sequence of the hydrophilic C-terminal domains are variable (e.g. see Fig. 2).

The conserved N-terminal regions contains four hydrophobic segments that are thought to span the membrane as α-helices, although these are not as long or as hydrophobic as those found in many other membrane proteins. Current models place the N terminus and the variable C-terminal domain in the cytoplasm.

Protease studies show the conserved N-terminal domain is relatively resistant to digestion, consistent with the notion that this part of the molecule is buried in the membrane. Most of these studies have been confined to Cx32. They show that extensive trypsin digestion cleaves the N-terminal domain into two ~10 kDa fragments, each fragment containing two of the putative transmembrane helices (Nicholson et al., 1981; Hertzberg et al., 1988a; Zimmer et al., 1987). This cleavage pattern is consistent with the disposition of the protein in the membrane as four transmembrane segments (Fig. 2). Immunocytochemical localisation studies using anti-peptide antibodies provide further evidence for the proposed orientation and disposition (Milks et al., 1988).

The structural predictions based on connexin sequences have been influenced by the supposition that connexins are the components of connexons, but can connexins, folded in the predicted way (or another way), be accommodated in the accepted connexon model? The N-terminal conserved regions can be fitted into the model as can any other polypeptide of about 18,000 Da with four potential transmembrane segments arranged as a four-α-helical bundle (Milks et al., 1988). There is, however, no space for the variable cytoplasmic tail. This dilemma has been largely ignored on the assumption that this domain of the connexin polypeptide is disordered and therefore is invisible to the imaging techniques used (Makowski, 1988; Tibbits et al., 1990; Sosinsky, 1992). But is such an assumption justified?

This premise can be tested by calculating (Fig. 3) the dimensions of the cytoplasmic domains (the *disordered regions*) for Cx32 and Cx43 to see if they might reasonably be invisible.

The transmembrane segment of each subunit arranged as a four-α-helical bundle, with a diameter of 20 Å and a length of 75 Å, can accommodate a protein mass of about 18 kDa (allowing for extracellular loops and based on an average density of 0.77 Da/Å^3; Makowski, 1988). The protein mass that must lie on the cytoplasmic face is therefore

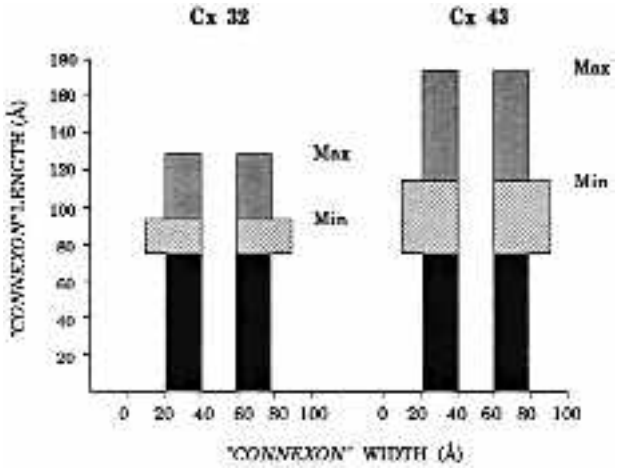

Fig. 3. Calculated lengths of connexons formed from Cx32 and Cx43 with extended or compact cytoplasmic domains. The 75 Å long transmembrane region of a connexin, arranged as four α-helices, accounts for 18 kDa of protein. A connexon assembled from six such 18 kDa subunits would have the dimensions shown in black. The extra mass (14 kDa for Cx32 and 25 kDa for Cx43) is believed to extend into the cytoplasm. The length of the cytoplasmic protrusion will depend on its structure. The dimensions of connexons made from Cx32 or Cx43 with cytoplasmic domains in an extended or compact structure are shown in dark grey and light grey, respectively. For further details, see text.

about 80 kDa for a hexamer of Cx32 and 150 kDa for a hexamer of Cx43. If the cytoplasmic domain of each connexin forms an extended cylinder with the same diameter as the membrane domain, *connexons* formed from Cx32 or Cx43 would be approximately 130 Å or 185 Å in length, respectively (Fig. 3), generating junctional complexes with minimum thicknesses of 26 nm or 37 nm (compared to the reported value of 15 nm; Caspar et al., 1977; Unwin and Ennis, 1984; Hoh et al., 1991). If, however, the protein from all the subunits collapses (proposed by Sosinsky, 1992, to explain the absence of cytoplasmic mass in shadowed and freeze-etched images) and spreads into a compact disk structure on the cytoplasmic face of 80 Å in diameter (this value being the maximum allowed by taking the minimum centre-to-centre spacing of the connexons in the gap junction; Baker et al., 1985), overall lengths would be 95 Å or 115 Å for the same two connexins (Fig. 3), generating a minimum junctional thicknesses of 19 or 23 nm.

It is difficult to believe that this extra mass, whether compacted or extended and which is equivalent in amount to 60% of the visible connexon for Cx32 (110% for Cx43), is missed by all the various imaging techniques used to date, including techniques where possible disordered domains should not affect visualization (i.e. thin-section TEM, atomic force microscopy and freeze-fracture).

But why should the cytoplasmic domains be disordered? The very nature of isolated gap junctions as paracrystalline arrays of closely packed connexons should constrain movement and impose at least some limited order on the cytoplasmic regions of connexins, making them apparent (perhaps at reduced resolution) in the processed image derived from those techniques that depend on structural repetition. By comparison, the acetyl choline receptor has a diameter similar to that of the connexon and a mass similar to that of a hexamer of Cx43, and its full length of approximately 120 Å (see Unwin, 1993) can be seen by the techniques used to examine gap junctions.

Further evidence against a large cytoplasmic extension comes from proteinase studies. The cytoplasmic domains of Cx32 and Cx43 are both removed by proteinase treatment. However, proteinase-treated liver and heart gap junctions, seen by negative staining (Caspar et al., 1977; Baker et al., 1985; Yeager and Gilula, 1992), thin-section (Sosinsky et al., 1988; Yeager and Gilula, 1992), atomic force microscopy (Hoh et al., 1991) and X-ray diffraction (Makowski et al., 1984), appear much the same as control specimens. Whilst each technique has its own inherent limitations, it is difficult to reconcile the uniformity of the results with the current model of connexin disposition. For example, negative staining reveals the water-accessible surfaces of gap junctions and one would expect to see substantial changes in the deposition of the stain if large cytoplasmic domains had been removed. Similarly, atomic force microscopy measures width directly in solution and the absence of any change in liver gap junction width (14.5 nm) after trypsin treatment, under conditions where Cx32 has lost one third of its total mass, is difficult to understand (Hoh et al., 1991).

Clearly, even in the absence of an alternative candidate protein, there are severe problems in relating the proposed structure of connexins to connexon architecture.

STRUCTURE OF DUCTIN

Ductin is present in all cells whether or not they form gap junctions, as it is also the major component of the proton channel of the vacuolar H^+-ATPase (V-ATPase: Mandel et al., 1988; Nelson, 1992). A discussion of the possible evolution of ductin function from a proton channel to a gap junction channel is described elsewhere (Finbow et al., 1991).

The sequence of ductin has now been determined from fungi, plants and animals and it is highly conserved (~80%) across the evolutionary spectrum (Holzenburg et al., 1993). Ductins from different species are of similar size (150-160 residues) and all have four extended hydrophobic segments, each 25-30 residues in length (Fig. 2). The mass and primary structure of ductin are therefore highly compatible with the predicted structure of the connexon subunit polypeptide.

Ductin is essentially a tandem repeat of two 8 kDa domains and each domain has homology with the 8 kDa subunit c of the F_1F_0 ATP synthase (Mandel et al., 1988). This relationship is not unexpected as the V-ATPase probably arose from a progenitor F_1F_0-like ATP synthase of archaebacteria (Gogarten et al., 1990).

Ductin in its gap junction form is remarkably resistant to proteinases (Finbow et al., 1992). Pronase treatment has no detectable effect on the junctional morphology and little effect on the protein (e.g. in treated *Nephrops* junctions only five N-terminal amino acids are removed and the rest of the

polypeptide remains intact), suggesting that the protein is largely buried in the junctional membranes. The accesibility of the N terminus to Pronase and also to N terminus-directed antibodies (Finbow et al., 1993), suggests that this part of the polypeptide is located on the exposed surfaces of the isolated junctions (which would be the cytoplasmic faces in situ). The sequence of ductin can be arranged as four transmembrane α-helical segments (Fig. 2). These segments and the two loops that are predicted (from the cytoplasmic location of the N terminus) to lie on the extracellular face (loop 1-2 and loop 3-4, see Fig. 3) show a very high degree of conservation. The conservation of these loop regions is consistent with them being sites of protein-protein interaction, which would explain the observation that gap junctions can form between cells of different species (e.g. *Xenopus* and hamster; Pitts, 1977). These loops are also the predicted site of interaction with other subunits of the V-ATPase (Finbow et al., 1992, 1993). In contrast the N and C termini and the loop predicted to lie on the cytoplasmic face (loop 2-3), are poorly conserved although this loop and the N terminus almost always contain acidic residues.

Such a disposition of the protein in the membrane is consistent with its insensitivity to proteases and to the reactivity of the glutamic acid residue in the fourth putative transmembrane segment to the lipophilic reagant *N*,*N*′-dicylohexyl carbodiimide (DCCD). This reagent irreversibly inhibits proton pumping of the V-ATPase (Arai et al., 1987) and has recently been shown to block metabolic cooperation and dye transfer between cells (Finbow et al., 1993), both of which are believed to be mediated by gap junctions. DCCD likewise blocks proton translocation of the F-type ATP synthase and again the reagent binds to an acidic residue in a transmembrane domain of the 8 kDa subunit c of the F_0 sector.

Freeze-fracture electron microscopy reveals an abundance of gap junctions in the hepatopancreas of *Nephrops norvegicus* and sufficient (ductin containing) gap junctions can be isolated for the application of such techniques as Fourier transform infrared (FTIR) spectroscopy, which can be used to determine the type and proportion of secondary structure. Such spectroscopy applied to *Nephrops* gap junctions (Holzenburg et al., 1993) reveals an α-helical content of ~60%, a value in close agreement with the prediction (from the sequence) that ductin has four transmembrane α-helices. In summary, the experimental data (proteinase resistance, antibody accessibility, chemical labelling and spectroscopic analysis) and secondary structure predictions all suggest four transmembrane α-helices.

Image reconstructions produced from negatively stained *Nephrops* gap junctions, reveal the unit cell to be a hexamer of 16-18 kDa subunits arranged around a central stain-filled channel (Holzenburg et al., 1993; and Fig. 1f). A separate study has been carried out on gap junctions isolated from the hepatopancreas of the closely related species *Homarus americanus* (Sikerwar et al., 1991). This shows essentially the same features, although the identity of the protein in this case was unknown (more recent data show that the major protein component of *Homarus* gap junctions is ductin; Holzenburg et al., 1993). The proton channel form of ductin may have a similar structure. Stoichiometric studies (Arai et al., 1988) on the V-ATPase show that six copies of ductin are present per enzyme complex and, furthermore, the F_1 sector of the F_1F_0 ATP synthase has pseudo sixfold symmetry (Boekema et al., 1988; Abrahams et al., 1993) along the same axis as the ductin hexamer (i.e. perpendicular to the membrane).

A computer-generated model of ductin has been created (Finbow et al., 1992; and Fig. 1b, c and e) and whilst caution must be adopted in the interpretation of such models, the experimental evidence and the severe constraints imposed by the intrinsic demands of multipass transmembrane proteins leave little freedom in the choice of design. The model has been built as a four α-helical bundle. The structure of a number of proteins based on four helical bundles has now been solved, providing a comparative basis for the model (see Cohen and Parry, 1990). In such bundles the α-helices are often arranged antiparallel and at a tilt to one another. Arranging ductin as a four-helical bundle is also consistent with the tandem repeat of two 8 kDa hairpins, as helices 3 and 4 in such a bundle occupy a symmetrical location with respect to helices 1 and 2 (see Fig. 12 of Finbow et al., 1992). Such an arrangement for ductin results in a discontinuous hydrophilic surface on helix 1, which, if the protein were a monomer, would face the hydrophobic core of the bilayer. The remaining external faces of the bundle, throughout a central 40-50 Å region, are hydrophobic. Taking six ductin subunits and placing them symmetrically around a central sixfold axis, with helix 1 to the centre, creates a channel of approximately 15-20 Å in diameter (Fig. 1e). The amino acid residues exposed on the surface of this channel are small or hydrophilic (e.g. serine, threonine, glycine and alanine residues) interspersed with two bands of valine (Fig. 1c). Such a surface is compatible with the channel being filled with water. A continuous surface of hydrophobic residues might tend to exclude water and charged residues might block the channel by reducing the mobility of the water molecules through formation of hydration shells. Taking into account helical packing considerations between neighbouring subunits, the minimum average distance of helices from the centre of the channel is 20 Å.

The shape and dimensions of the hexameric ductin model are very similar to the re-constructed image produced from electron micrographic data (Fig. 1; and Holzenburg et al., 1993). The minimum length of the *Nephrops* ductin hexamer in the model is ~65Å. Such a length is consistent with the width (14-15 nm) of isolated *Nephrops* gap junctions measured in thin-section electron micrographs (Leitch and Finbow, 1990).

The model of the ductin-based channel is derived primarily from studies on *Nephrops* (crustacean) gap junctions. Ductin is highly conserved (>85% identity between *Nephrops* and rodent; Finbow et al., 1992; Nezu et al., 1992) and ductin-based gap junctions prepared from rodent liver have the same morphological appearance as those prepared from *Nephrops* (Finbow et al., 1983; Finbow and Meagher, 1992). It is also known from proteinase resistance and antibody accessibility studies that the disposition of ductin in rodent gap junctions is very similar to that in *Nephrops* junctions (Finbow et al., 1993). Therefore the model generated for the *Nephrops* ductin can be compared

not only with other models for arthropod junctions, but also directly with the accepted model of the rodent connexon.

The ductin channel fits very well into the accepted model of the connexon (Fig. 1). Given that the ductin is found in preparations of rodent liver gap junctions made by standard procedures (Finbow et al., 1986; Finbow and Meagher, 1992; Hertzberg et al., 1988b; Willecke et al., 1988) and the difficulty of relating connexin structure to the connexon model, it is reasonable to suggest that the presently accepted structure of the connexon is primarily based on ductin.

CONNEXINS, DUCTIN, GAP JUNCTIONS AND CELL-CELL COMMUNICATION

The above conclusion is independent of any role ductin or connexins may or may not have in cell-cell communication. It is based primarily on a comparative analysis of the candidate proteins and the structure of highly ordered, isolated gap junctions. It is not known how closely related the structure and composition of these isolated gap junctions might be to active gap junctions in intact tissues.

It has been suggested (Berdan and Gilula, 1988; Dermietzel et al., 1989) that gap junctions composed exclusively of ductin form spontaneously from vacuolar membranes during isolation. If this were correct then the model of the connexon may have little relevance to the channels, perhaps formed from connexin, involved in cell-cell communication. However, such spontaneous generation seems unlikely. It would require abnormal fusion of vacuoles (which contain about 10 V-ATPase complexes per vacuole; Winkler and Westhead, 1980) to provide sufficiently large numbers of ductin channels. Secondly, it would depend on the aggregation of the ductin complexes into hexagonal arrays. And, thirdly, it would require precise pairing of arrays by either chance association of single membranes of the same size and shape, or by subsequent destabilisation and loss of unmatched areas. On these grounds it seems reasonable to assume that ductin-based gap junctions are not artefacts but are derived from normal cellular structures. The only recorded structures showing the same basic features are cell surface gap junctions.

Such an assumption is vindicated by structural studies on gap junctions in plasma membranes fractions (Sikerwar and Unwin, 1988) and on images of gap junctions from freeze-fracture replicas (Rash and Yasamura, 1992; Hirokawa and Heuser, 1992). These studies show that the connexons in gap junctions in situ have the same basic features in terms of shape and dimensions as the channels in the modelled junctions and the ductin hexamer complex.

If ductin is the basic unit of construction of the connexon at the cell surface, then what role do connexins have? As mentioned at the beginning of this Commentary, there is a wealth of evidence demonstrating that connexins are intimately associated with gap junctions and have an essential function in gap-junctional communication.

A step towards understanding the function of connexins would be a better knowledge of their structure.The recent crystallisation of Cx32 (Stauffer et al., 1991) may give a clue to the organisation of the oligomeric complexes formed from connexins. The dimensions of the unit cell of these crystals are 90 Å × 150 Å, where the shorter dimension is thought to represent the diameter of a connexin hexamer and the longer dimension the length (across the bilayer) of two hexameric units associated end to end. Such dimensions suggest that the hydrophilic C-terminal tail is incorporated radially in extra width; that is, in regions proposed by present thinking to be within the membrane. However, oligomeric complexes of Cx32, isolated from gap-junction-enriched fractions (Stauffer et al., 1991), contain a pore that is similar to the proposed axial pore in the ductin hexamer complex. Connexins may therefore form channels of cell-cell communication that have a different structure to that of the accepted model of the connexon.

Cell surface gap junctions could, in principle, be mixtures of channels formed from connexins or ductin, although the morphological data suggest that the connexons are uniform and the antibody blocking data (antibodies to either connexins or ductin inhibiting all detectable communication; see above) appear to exclude two alternative channel pathways.

Alternatively, the connexins may be essential components involved in the assembly, maintenance, regulation or degradation of vertebrate gap junctions but be difficult to visualize by available imaging techniques (as are some components of adhesive junctions). If connexins do fulfil such roles it seems likely that equivalent molecules must have evolved to provide related functions in invertebrates.

Genetic analysis (Macdonald, 1985) and expression studies (see above) suggest that the formation of gap junctions and the control of junctional communication involves multiple gene products. This is perhaps to be expected given the complex patterns of cell-cell communication that have been observed in vivo; patterns that change, spatially and temporally, during development and in various diseased states (for review see Pitts et al., 1988). Some of the molecules that control these patterns have now been identified (e.g. cadherins, Mege et al., 1988; and wnt1, Olsen and Moon, 1992) and it seems possible that connexins, an interestingly complex family of tissue-specific molecules, may have some comparable role.

The main plank of this Commentary has been the use of structural analysis to relate ductin to the channel of the isolated gap junction, a structure so elegantly portrayed by the first imaging studies in 1977. However, care must be taken in equating this model of the isolated junction to the active gap junction at the cell surface, as functionally important components may be lost during the preparation of core structures with sufficient crystallinity for imaging studies. A better understanding of gap junctions and cell-cell communication awaits more detailed information on the organization of active junctions in situ and on the structure of connexins.

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