

## Invited Review

# Gap junction channels: new roles in disease

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**Summary.** The importance of intercellular communication to complex cellular processes such as development, differentiation, growth, propagation of electrical impulses and diffusional feeding has long been appreciated. The realization that intercellular communication is mediated by gap junction channels, which are in turn comprised of a diverse family of proteins called the connexins, has provided new tools and avenues for studying the role of intercellular communication in these important cellular processes. The identification of different connexin isoforms has not only enabled the development of specific reagents to study connexin expression patterns, but has also allowed the functional properties of the different connexin isoforms and how they interact with each other, to be explored. Increasingly, the knowledge gained from studying connexin diversity is being used to investigate the role played by gap junction channels in a number of diseases. In this article we highlight selected cases where gap junction channels have been shown or are believed to be directly involved in the disease process.

**Key words:** Connexin, Cancer, Cataract, CMTX-disease, Heart disease

## Introduction

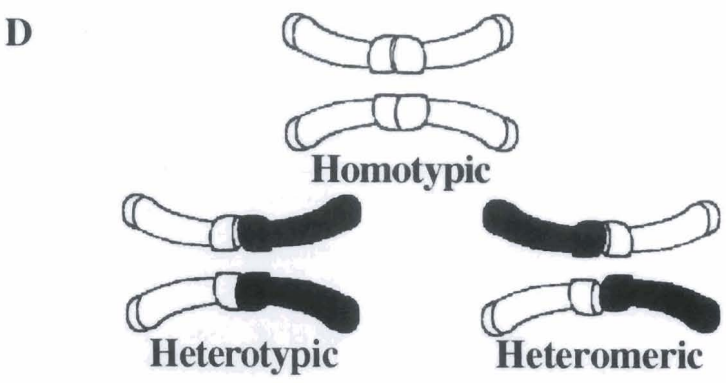
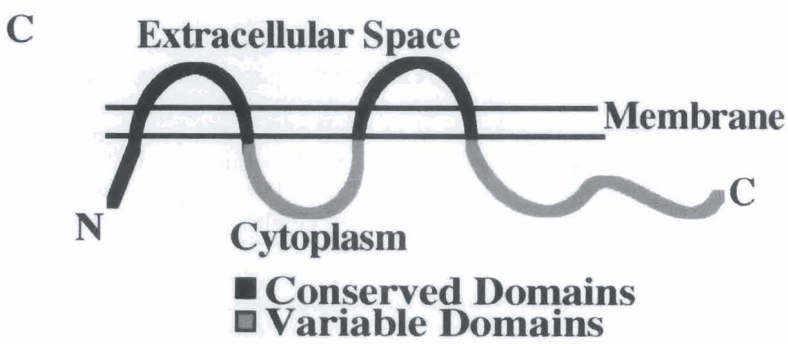
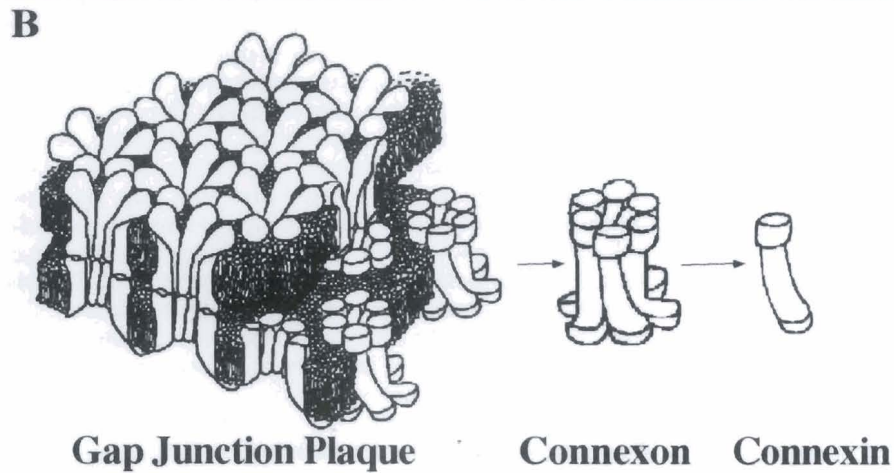
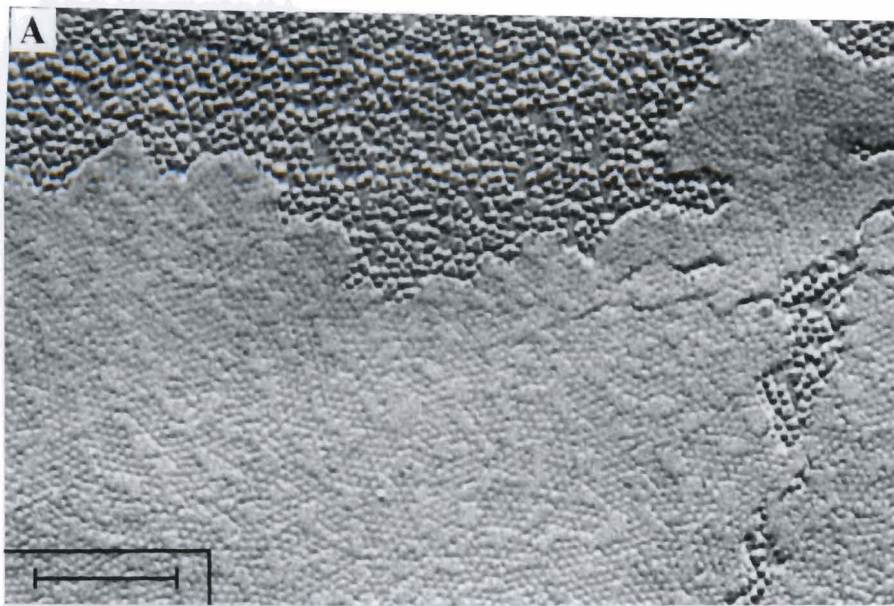
30 years after Loewenstein (1981) first proposed that intercellular communication was mediated by gap junctions, the functional significance of the diversity of the connexins which form the gap junction channels, has become a major research focus (Hall et al., 1993; Kanno et al., 1995). By providing a direct pathway for the diffusion of ions and small molecules between the cytoplasm of adjacent cells, connexins have been implicated in the regulation of cell development, growth and differentiation; propagation of electrical impulses in excitable tissues; and signalling and diffusional feeding

in avascular tissues. However, despite considerable correlative studies supporting a role for gap junction channels in these important processes, experimental evidence linking gap junction channels directly to these processes has to date been limited. Fortunately, recent efforts to probe the functional significance of connexin diversity coupled to findings that link gap junction dysfunction with disease, are beginning to provide valuable insights into the important role played by gap junction channels in these cellular processes. In this review, we summarize recent progress in elucidating the role of connexin diversity and highlight selected cases where certain connexin isoforms have been implicated in specific disease processes.

## Gap junction channel structure

Gap junctions are observed where the membranes of adjacent cells come into close apposition (Revel and Karnovsky, 1967). Classically, gap junctions appear in freeze fracture images as a dense array of particles in the plasma membrane of one cell with a mirror image of pits occurring in the membrane of the adjacent cell (Chalcroft and Bullivant, 1970) (Fig. 1A). The structural unit of the gap junction channel is the connexon or hemi-channel (Fig. 1B) (Makowski et al., 1977). Each connexon is comprised of six connexin polypeptides which oligomerize to form an aqueous pore that spans a single plasma membrane. To form a complete gap junction channel, two connexons from adjacent cells align and dock with each other to form a continuous channel linking the cytoplasm of the two cells. Classic gap junction channel permeability studies indicate the pore formed by adjacent connexons is 0.8 to 1.4 nm in diameter, is relatively non-selective and allows the passage of molecules up to 1200 Daltons (Makowski et al., 1977; Simpson et al., 1977; Flagg-Newton et al., 1979).

This picture of an ubiquitous, large non-selective channel was shattered by the finding that the connexins (Cx) are in fact a diverse family consisting of at least 14 different isoforms (Willecke et al., 1991; White et al., 1995a; Kumar and Gilula, 1996). These proteins share a



**Fig. 1:** Gap junction channel structure. **A.** A classical freeze-fracture image of a gap junction plaque showing E-face pits and P-face particles. Scale bar: 0.1 $\mu$ m. **B.** Molecular model of gap junction channel structure. **C.** Proposed connexin membrane topology highlighting conserved and variable domains. **D.** Probable connexon configurations.



common molecular topology crossing the membrane 4 times with the N and C termini being located in the cytoplasm (Fig. 1C). In addition, they exhibit a number of highly conserved regions with most sequence variation occurring in the cytoplasmic loop and tail of the molecule. Furthermore, although conserved between species, connexin expression has been shown to be tissue and cell specific, with some cell types expressing multiple connexin isoforms. This multiple expression of connexins raises the possibility that hybrid channels could exist between adjacent cells (Fig. 1D). Experimental evidence suggests two different hybrid configurations are possible: heterotypic cell-to-cell channels in which each connexon or hemichannel consists of a specific connexin isoform (Bukauskas et al., 1995; Venance et al., 1995); or heteromeric channels where each connexon is a mixture of the different connexin isoforms expressed in a particular cell type (Stauffer, 1995; Jiang and Goodenough, 1996).

### The functional diversity of connexins

As the cloning of the different connexin isoforms nears completion, the emphasis in gap junction research has shifted to determining the extent and functional consequences of connexin diversity. Connexin isoform distribution can be determined in a specific cell type or tissue at either the RNA or protein level using Northern analysis and immunolabeling techniques, respectively (Fig. 2I, II). This has been facilitated by the production of specific reagents (cDNA probes; PCR primers; anti-peptide antibodies) which enable the expression pattern of a particular connexin isoform to be studied in detail.

Functional studies of gap junction channels have traditionally used intercellular dye transfer and electrical measurements to probe the biophysical properties of cell-to-cell channels in a variety of cell types (Fig. 2III, IV). However, since we now know particular cell types are capable of expressing multiple connexin isoforms, it has proven difficult to associate junctional properties exclusively to a specific connexin isoform. To circumvent such difficulties, the gating and permeability properties of the individual connexins have been studied by using exogenous expression systems. These include the transient expression of connexins in paired *Xenopus* oocytes (Dahl et al., 1987; Swenson et al., 1989) and the establishment of stably transfected cell lines which express individual connexins (Eghbali et al., 1990).

Using these model systems in combination with dye transfer and electrical measurements researchers have firmly established that the individual connexins exhibit differences in regulation by both voltage and phosphorylation, and have different single channel conductances (see Kanno et al., 1995). However, while being extremely useful parameters with which to categorize the different connexins, these parameters tell us little about the physiological consequences of connexin diversity. Since a major role of gap junction channels is the exchange of intracellular second

messenger molecules between interconnected cells (Sáez et al., 1989), a more appropriate biophysical parameter of connexin diversity is cell-to-cell channel permeability.

A recent series of experiments has addressed this issue, using a variety of substrates that exhibited slight variations in size, charge and/or conformation (Brissette et al., 1994; Steinberg et al., 1994; Elfgang et al., 1995; Veenstra et al., 1995). Surprisingly, the channel forming regions of the different connexins did not act as simple passive pores but exhibited connexin specific differences in cation and anion permeabilities (Veenstra et al., 1995). Such differences in connexin permeability could permit discrimination between known intracellular second messengers producing connexin specific differences in the intercellular propagation of signalling pathways.

Another feature of connexin diversity has been revealed by the recent observation that some pairs of connexins do not form functional gap junctions when each is expressed in adjacent cells (Bruzzone et al., 1993; White et al., 1994, 1995b; Elfgang et al., 1995). Thus the expression of incompatible connexins could lead to the establishment of communication compartments (Paul, 1995). Evidence for the involvement of incompatible connexins in the formation of communication compartments has been shown recently in the heart (see later; Bruzzone et al., 1993) and in early embryo development (Dahl et al., 1995).

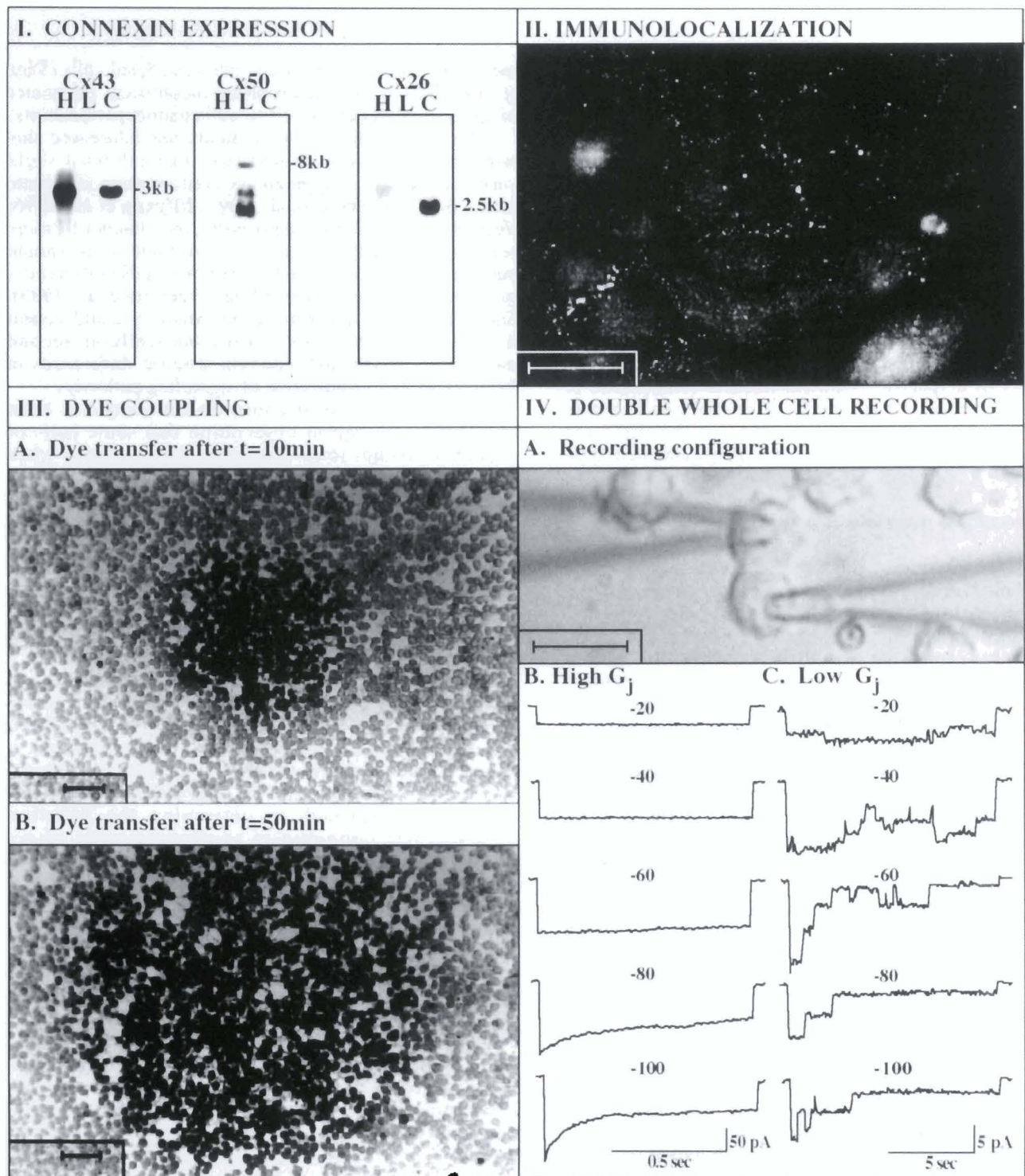
While this molecular physiological approach has proven to be an invaluable method for determining the functional significance of connexin diversity, it can only provide limited information on the involvement of specific connexins in dynamic multicellular processes such as development, growth, and differentiation. An alternative approach to determine the function of a specific connexin is to observe what effect the selective inhibition of a particular connexin isoform has on such processes. This has been achieved in a variety of ways including the injection of connexin specific antibodies (Becker et al., 1995) and the expression of a dominant negative connexin mutation (Paul et al., 1995), both of which lead to consistent developmental defects, or the targeted knockout of a specific connexin gene (Reaume et al., 1995). These approaches, particularly in combination with subsequent physiological studies, offer considerable promise for our understanding of intercellular communication, its involvement in maintaining normal tissue function, and its role in disease processes.

### Functional role for connexins in disease processes

Studies of a number of diseases have implicated members of the connexin family, revealing new roles for the connexins and raising new questions about how their activity is regulated. Below we highlight the role connexins play or are believed to play in cancer, cardiac disease, peripheral neuropathy, and lens cataract formation.



## Gap junctions and disease



**Fig. 2.** Methods used to study gap junction channels. **I.** Connexin expression. Total RNA isolated from heart (H), lens (L) and cornea (C) is probed with cDNAs encoding for connexins 43, 50 and 26. **II.** Immunolocalization. Cx43 antibodies are localized to membrane appositions between adjacent lens epithelial cells, forming a punctate labeling pattern. Scale bar: 20 $\mu$ m. **III.** Dye coupling. The intracellular injection of neurobiotin is used as a gap junctional tracer in the rat mammary tumor cell line BICR M1R<sub>k</sub>. The extent of dye spread is visualized after fixing the cells and processing with horseradish peroxidase conjugated to streptavidin (Vaney, 1991). A) Cells fixed 10 minutes after injection. B) Cells fixed 50 minutes after injection showing more extensive dye spread. Scale bar: 200 $\mu$ m. **IV.** Double whole cell recording. A) Two patch pipettes are shown attached to a pair of lens epithelial cells. Scale bar: 20 $\mu$ m. By using the whole cell recording technique membrane voltages can be independently clamped in both cells and the current flow via the gap junction channels recorded (Neyton and Trautmann, 1985). B) Macroscopic junctional currents recorded from lens epithelial cells show voltage dependence at transjunctional voltages greater than -60mV. C) Single gap junction channel currents can be resolved after spontaneous uncoupling of lens epithelial cells.

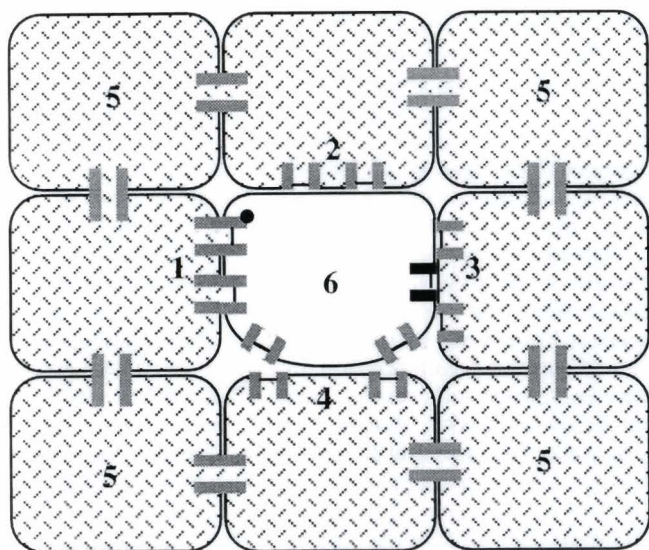


### i) Cancer - Connexins as tumor suppressors

The initial finding that gap junction channels form an intercellular pathway for large molecules was immediately followed by the suggestion that these channels play a role in the regulation of normal growth control and in cancer (Loewenstein, 1979). This initial hypothesis stated that within a tissue there is a pool of signal molecules, which are distributed and equilibrated between the cells via gap junction channels. Disruption of this communication pathway alters the concentration of the signal molecules in some of the cells and, therefore, may lead to abnormal growth. However, since the signal molecules involved in this process remain to be identified, we still have no direct proof for this hypothesis. This initial hypothesis has been subsequently modified by Yamasaki and coworkers (Mesnil and Yamasaki, 1993) to explain early tumor development. They proposed that, within a population of freely communicating cells, certain cells are genetically primed to become tumorigenic. Intercellular communication between these primed cells and those in the surrounding normal tissue is critical in maintaining the normal growth rate of the primed cells. If this communication is disturbed, these primed cells may escape the growth control and clonally expand. These tumor cells may themselves be well coupled to each other, thereby forming a communication compartment of their own.

There are several ways by which this could happen (Fig. 3):

1) By closure of connexons in the membrane. Some connexins are known to be targets for the products of oncogenes such as *src*, *ras* and *gag* and a variety of



**Fig. 3.** Schematic diagram summarising the involvement of gap junction channels in cancer (see text for details). 1: connexon closure; 2: reduced expression; 3: incompatible connexins; 4: reduced cell adhesion; 5: normal coupling; 6: cancer cell.

tumor promoters such as phorbol esters, which act by reducing junctional permeability by direct phosphorylation of the channel protein (Atkinson and Sheridan, 1985; Klaunig and Ruch, 1990; Kurata and Lau, 1994; Loo et al., 1995).

2) By alteration in connexin expression. In some liver carcinomas the expression of Cx26 and Cx32 is permanently decreased (Sakamoto et al., 1992; Mesnil et al., 1993). Tumor cells are also reported to change their pattern of connexin expression during various stages of tumor development (Budunova and Slaga, 1994; Oyamada et al., 1995) and connexin43 has even been proposed as a tumor marker in hepatocarcinomas (Sugie et al., 1995).

3) By expression of incompatible connexins. The recent results on the compatibility of connexins to form communicating heterotypic channels indicate this may be a key mechanism which allows the disruption of heterologous coupling while maintaining cell-cell communication within the neoplasm.

4) By reduced cell adhesion. Junctional communication may also be affected by indirect mechanisms such as the lack of cell adhesion molecules (Mesnil and Yamasaki, 1993).

The above evidence suggests that connexins function to suppress tumor formation. This notion has recently gained support from a study which utilized subtractive hybridization techniques to isolate putative tumor suppressor genes. One of the isolated genes was found to encode for Cx26, a major liver gap junction protein (Lee et al., 1991). Furthermore, restoration of gap junctional communication by anti-tumor promoting agents such as retinoids leads to the restoration of normalized growth rates and growth patterns in some tumor cells (Mehta et al., 1989). However, the most convincing evidence for a tumor suppressor function of connexins arises from recent experiments where transfection and over-expression of connexins in communication deficient tumor cells resulted in restoration of growth control *in vitro* and the loss of tumorigenicity *in vivo* (Mehta et al., 1991; Zhu et al., 1992; Rose et al., 1993). Again, this effect seems to be dependent upon the connexin type expressed. Expression of the endogenous Cx43 is effective in growth retardation of C6 glioma cells *in vitro* and *in vivo* (Naus et al., 1992; Zhu et al., 1992) whereas the non-endogenous Cx32 is ineffective. However, transfection with both connexins established a communication competent phenotype within the tumor cells (Bond et al., 1994).

In summary, it would appear that studies into early tumor development have provided new evidence for the involvement of the connexins in the control of growth. Further rapid progress in this area can be expected as the physiological properties of the different connexin isoforms are elucidated and incorporated into models for tumor development in different tissues.



*ii) Heart disease-arrhythmias and hereditary cardiac malformations*

Gap junctions form the low resistance pathways for electrical conduction from myocyte to myocyte in the heart, coordinating their contraction (Severs, 1995). Cx43 is the major component of gap junctions in the working myocardium, with Cx40 the major component of the fast conducting system (bundle of His, bundle branches and Purkinje fiber network) (Gourdie et al., 1993). Cx45 is also said to be present in cardiac tissues (Kanter et al., 1993). By means of the *Xenopus* oocyte expression system it has been established that connexins 43 and 40 do not form functional heterotypic channels (Bruzzone et al., 1993) and in regions where the Purkinje fibers turn into the functional myocardium both Cx40 and Cx43 are expressed, presumably forming parallel, homotypic gap junction channels in this area. The rest of the fast conduction system is effectively a communication compartment quite separate from the working myocardium. Cardiac tissues, therefore, have a distinct spatial distribution of gap junctions and it has become increasingly clear that perturbations to gap junction expression, distribution and function play a major role in cardiac disease.

During development, gap junctions occur entirely around the embryonic, cigar shaped myocytes, providing a degree of side-to-side coupling (transverse propagation). In humans this distribution pattern persists until about 5-6 years of age (Peters et al., 1994b) and it is only later that the junctions become confined predominantly to the intercalated disks of the more cylindrical shaped adult myocyte (Fig. 4A) and electrical propagation follows the longitudinal axes of the cells. This maturation is achieved some time after postnatal hemodynamic changes (for example peak systolic stress in the ventricular wall at 2-3 years of age) and may be important in evaluating long-term effects following surgical correction of congenital heart defects. The postnatal changes in gap junction distribution may provide an explanation for long-term postoperative arrhythmias which can develop many years after such surgery (Spach, 1994). In the adult heart, intercalated disks at the ends and on branches from the side of the myocyte mean each cell has about 9 or 10 intercalated disk connections with neighbouring cells, each cell having up to about 1000 gap junctions in total. The exact arrangement of these is considered to be vital in maintaining efficient cardiac function. Since a similar decrease in transverse propagation to that which occurs in the embryonic ventricles also occurs in the atria with aging (Spach, 1994), and may be occurring with remodelling at healed ventricular infarct border zones (Smith et al., 1991), it has been suggested that a major adaptive structural response of cardiac muscle is a progressive loss of side-to-side electrical connections (Spach, 1994). Certainly, a major rewiring of conduction patterns of the ventricle occurs during development and the result of aberrations to the pattern, such as the

congenital accessory atrioventricular pathways of Wolff-Parkinson-White syndrome, can be attributed to the abnormal gap junctional communication routes (Peters et al., 1994a).

A result of cardiac ischemia is the formation of fibrotic infarct zones. Around the edges of these zones gap junction distribution is highly perturbed (Fig. 4B) and crossing the infarct, thin fiber bridges are seen with extensive gap junction connections between the few remaining fiber cells (Smith et al., 1991; Green and Severs, 1993). It is no surprise then that reentry arrhythmias have been mapped to these regions (Dillon et al., 1988) and that sudden death resulting from ventricular tachyarrhythmias in healed infarct patients appears to occur spontaneously (Saffitz, 1994). It may require only the loss of one or two cells in these regions, or slightly altered coupling levels between them, to have quite devastating effects (Smith et al., 1991). Furthermore, the total content of Cx43 is reduced in apparently normal ventricular myocardium away from the infarct itself, a feature also of cardiac hypertrophy (Peters et al., 1993). The reduced Cx43 gap junction content almost certainly contributes to the abnormal impulse propagation in these hearts (Peters et al., 1993). Current evidence suggests that a reduction in Cx43 content may therefore be a general pathogenetic feature of cardiac disease (Severs, 1994) although changes in the expression of other connexin types may also contribute to altered electrophysiological function. In the atria too, fibrillation can be attributed to abnormal gap junction connections, and it has been proposed that rather than concentrating on drugs designed to target sarcolemmal ionic current channels, as is done with conventional pharmacological antiarrhythmic therapy, identification of ways in which the distribution of gap junctions in diseased cardiac muscle could be altered, might produce better therapeutic results (Spach and Starmer, 1995).

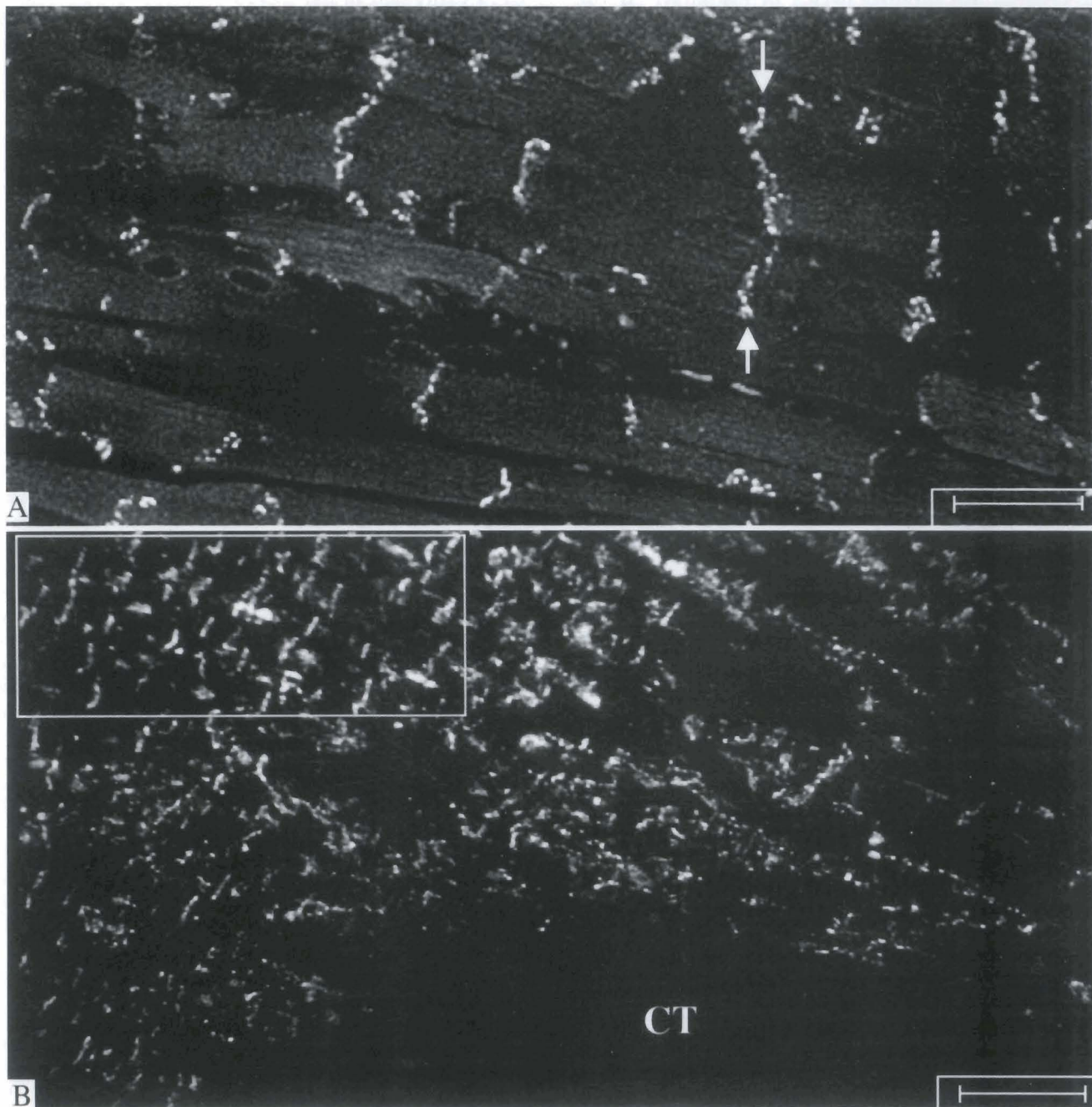
One method by which this could be achieved is by grafting. Fetal cardiomyocytes have been isolated from transgenic mice carrying a fusion gene of the alpha-cardiac myosin heavy chain promoter with a beta-galactosidase reporter and injected into the myocardium of syngenic hosts. The formation of stable grafts into the host heart was then followed using the beta-galactosidase marker (Soonpaa et al., 1994). Over the period of the experiments, nascent intercalated disks connecting the injected fetal myocytes to the host myocardium were formed. Although the unambiguous identification of gap junctional connections between the host and donor cells was not made, it is unlikely that the intercalated disks formed would not have had gap junctions. The potential for this procedure in establishing bridges across infarct zones, not only strengthening the heart wall, but more importantly, reestablishing electrical communication pathways, needs further exploration. The use of the connexin specific antibody probes provides one method for following subsequent gap junction distribution patterns.

Targeted knockout in mice of the Cx43 gene, one of



the most prevalent connexin proteins and the one expressed at the earliest stages of mammalian development, results in surprisingly normal development up until birth (Reaume et al., 1995). At birth, however,

swelling and blockage of the right ventricular outflow tract leads to hypoxia. These experiments would suggest that functional cardiac conduction does not uniquely require Cx43 channels and either other connexin types



**Fig. 4.** Cx43 immunohistochemical labeling of gap junctions in human adult ventricular myocardium. **A.** In normal adult myocardium the gap junctions are restricted predominantly to the intercalated disks joining the cells at their ends or at the ends of side branches (for example between the arrows). In contrast to embryonic heart tissue there is little evidence for lateral connections between cells. Scale bar: 25 $\mu$ m. **B.** With cardiac ischemia, cell death leads to the formation of fibrotic connective tissue infarcts (CT). Around the edges of these infarcts the normal gap junction distribution is severely affected; such regions are known to be sites of reentry arrhythmias. Away from the infarct region in the diseased heart the gap junction distribution appears relatively normal (boxed area) although quantitative analysis reveals an overall drop in the area per cell of Cx43 gap junction plaques present. Scale bar: 70 $\mu$ m.



become activated or coexpressed connexins are upregulated. Whether the heart could continue to function adequately in the adult mouse is not known of course, but it is known that mutations of the Cx43 gap junction gene in humans can lead to functional and developmental abnormalities in the heart (Britz-Cunningham et al., 1995). In this study, the Cx43 DNA from 25 normal subjects and 30 children with a variety of congenital heart diseases was compared. All six children with syndromes that included complex heart malformations had mutations of the Cx43 gene. Five of the affected children had a substitution of proline for serine at position 364 of the Cx43 amino acid sequence. That this was a significant alteration was tested by transfecting low communicating cell lines with a mutant Cx43 sequence with the same substitution. These cells failed to communicate, as compared to cells transfected with normal Cx43, indicating that reduced gap junctional communication can result from a single substitution to the Cx43 gene and such a defect can be directly associated with heart malformations and defects of laterality.

### iii) CMTX disease

Charcot-Marie-Tooth disease (CMT) is one of a group of peripheral neuropathies in which progressive myelin degeneration produces distal extremity weakness, atrophy, sensory loss and areflexia. The X-linked form (CMTX) of this disease has recently been directly linked to mutations within the Cx32 locus (Berghoffen et al., 1993). Subsequent sequence analysis of this coding region in CMTX disease patients from 39 families have identified 33 distinct Cx32 mutations to date (Berghoffen et al., 1993; Fairweather et al., 1994; Ionasescu et al., 1994; Orth et al., 1994; Bone et al., 1995). These mutations occur throughout the coding sequence and vary in nature ranging from truncations, deletions and frame shifts to single base pair substitutions indicating that virtually all regions of the molecule are important in its function. Furthermore, some families exhibited mutations in the noncoding regions of the Cx32 gene suggesting that promoter and splice sites may be responsible for causing the disease.

The finding that mutations in the Cx32 gene cause CMTX disease raises a number of questions. The first being: what role does Cx32 play in the Schwann cells? Immunolocalisation studies of Cx32 shows that Schwann cells express Cx32 and concentrate it in the uncompact membranes adjacent to the nodes of Ranvier and at the incisures of Schmidt-Lantermann (Berghoffen et al., 1993; Spray and Dermietzel, 1995). This distribution has led some workers to postulate that Cx32 in Schwann cells forms intracellular or reflexive gap junction channels (Berghoffen et al., 1993; Paul, 1995). These channels would connect the adjacent wraps of myelin at incisures and paranodal membranes providing a shorter radial pathway for diffusion of ions and nutrients between the Schwann cell body and its

distal processes. In this model, Cx32 mutations would disrupt this communication pathway and cause Schwann cell degeneration.

In an effort to directly determine the effect of mutations on Cx32 function, selected Cx32 mutants have been expressed in the paired *Xenopus* oocyte system (Bruzzone et al., 1994; Rabadan-Diehl et al., 1994). Of the 4 cases studied to date, 3 failed to form active intercellular channels, even though Cx32 protein accumulated at areas of cell-cell apposition. Therefore, these 3 mutations appear to have no effect on intracellular trafficking but most probably affect channel gating or assembly (Bruzzone et al., 1994). The fourth mutation tested formed active channels that displayed normal gating responses to pH and voltage even though most of its carboxy-terminal tail was deleted. Thus the precise role of the carboxy-terminal region remains to be defined but is apparently not related to channel assembly or gating by known stimuli (Rabadan-Diehl et al., 1994). Further analysis of these and additional CMTX disease-linked mutations should produce new insights into what regions of the protein are responsible for the different properties of the Cx32 channel.

Another question raised by the finding that Cx32 mutations cause CMTX disease, is why do Cx32 mutations not cause gross functional abnormalities in other organ systems? One explanation, as noted earlier, may be that many cells express multiple connexin isoforms which could compensate for the mutations. Because of this redundancy in connexin expression we would predict the specific effects of Cx32 mutations on the peripheral nervous system to occur because the myelin producing Schwann cells only produce Cx32. Or alternatively, if an additional connexin is produced, this connexin is unable to compensate for the non-functional Cx32 (Paul, 1995). A third possibility is that the expression of the mutant Cx32 gene product actively produces the disease phenotype, possibly by inhibiting other connexins expressed in Schwann cells. This possibility is supported by the finding that CMTX disease-linked mutations, when coexpressed with a normal connexin in the paired oocyte system dominantly inhibit communication (Bruzzone et al., 1994). As pointed out by Paul, this hypothesis could be tested by the production of a *bona fide* CMTX mutation in mice (Paul, 1995).

### iv) Connexins and lens cataract

The importance of intercellular communication is particularly obvious in avascular tissues such as the eye lens which rely on gap junctional channels for diffusional feeding. In this tissue, the anterior epithelial cell monolayer which interfaces with the aqueous humor, contains most of the transporters and ion pumps and controls homeostasis throughout the lens via an extensive network of gap junction channels (Goodenough, 1992). This concept of the freely communicating lens has been widely accepted as one of



the important fundamentals for tissue transparency, with loss of homeostasis leading directly to lens opacification. In view of the 'averaging' function of gap junctions, one would expect the loss of homeostasis and the resulting opacification to progress uniformly throughout the entire lens. This is, however, clearly not the case. In the early stages of the two most important cataract forms, senile cataract and diabetic cataract, opacities are locally confined and are embedded in seemingly normal tissue. At the histological level, fiber cells can be grossly swollen next to normal appearing cells. Hence, it appears possible that gap junction channels are not uniformly open but can close locally.

The syncytial properties of the normal lens are well supported by results from metabolic cooperation experiments (Goodenough et al., 1980) and whole lens electrophysiology (Mathias et al., 1981). The connexins expressed in the mammalian lens have been identified as Cx43 in the epithelial cell monolayer (Beyer et al., 1989), and Cx46 (Paul et al., 1991) and Cx50 (Kistler et al., 1985; White et al., 1992) in the fiber cells which make the bulk of the lens. That lens connexins form functional channels has been demonstrated by expressing them in *Xenopus* oocyte pairs (White et al., 1994) and by patch clamp analysis of isolated lens cells (Donaldson et al., 1994, 1995). There are other experiments, however, which indicate that the freely communicating lens model may be too simplistic. For example, dye spread between cortical fiber cells is not uniform but confined to subsets of fiber cells (Prescott et al., 1994). Dye transfer data is also inconsistent with the extensive epithelial-fiber cell coupling predicted by the model (Bassnett et al., 1994). Furthermore, gap junction channel gating differed between cortical and deeper lens regions. Acidification, a stimulus known to inhibit intercellular communication, results in an uncoupling of gap junction channels between cortical fiber cells but not those between fiber cells in the deeper regions of the lens (Baldo and Mathias, 1992). Together these results clearly show that the model of the freely communicating lens requires re-examination.

Post-translational cleavage of the carboxy-tail of Cx46 and Cx50 in maturing fiber cells (Kistler et al., 1990a,b) may explain the difference in pH sensitivity of gap junction channels in the outer and deeper lens regions. The outer cortical region which has gap junctions composed of full-length connexins, overlaps the region with pH sensitive gating, while deeper in the lens where the connexins are cleaved, uncoupling does not occur upon acidification. There is supporting evidence suggesting that the two phenomena are related but it is indirect at this time: experimental truncation of the carboxy-tail of Cx43 abolished low pH mediated closure of Cx43 cell-cell channels (Liu et al., 1993), and by analogy, in vivo cleavage of the lens fiber connexins 46 and 50 might have a similar effect. Obvious experiments to resolve this issue are to determine the cleavage sites in the Cx46 and Cx50 molecules and to carry out an electrophysiological analysis of truncation

mutants in transfected cells or oocytes.

Diabetic cataractogenesis is used here to support the possibility that gap junction gating may play a role in the cellular changes leading to lens opacification. Unexpectedly, the cellular changes caused by polyol accumulation and the resulting osmotic stress (Lee et al., 1995), appear spatially localized and, at least in the early stages, do not spread uniformly across the lens despite the abundance of gap junctions. In the diabetic rat model, cortical opacities occur due to increased light scatter in a zone about 100  $\mu\text{m}$  inwards from the lens periphery. This zone is characterized by massive tissue breakdown and liquefaction (Fig. 5A). It is confined to a band 50-100  $\mu\text{m}$  wide and surrounded, inwards and outwards, by normal appearing fiber cell tissue (Robison et al., 1990). Electron micrographs of the borders of the liquefaction zone reveal the co-existence of grossly swollen fiber cells immediately adjacent to normal appearing cells (Fig. 5B). The histopathology of these lenses can be comprehensively visualized using specific membrane labels in conjunction with confocal laser scanning microscopy (Bond et al., 1996). Equatorial sections labeled with fluorescein conjugated wheat germ agglutinin show the disruption of tissue by cell swelling and by the formation of liquid filled spaces (Fig. 5C). Again, the direct contact of grossly swollen fiber cells with apparently normal cells is evident. In the same section, Cx50 was labeled with rhodamine conjugated antibodies specific for the carboxy-tail of the molecule (Fig. 5D). It is evident that the zone of liquefaction lies within the cortical tissue containing gap junctions composed of uncleaved connexins which had previously been demonstrated to form functional gap junction channels (White et al., 1994; Donaldson et al., 1995).

These histopathological pictures are unexpected but could be readily explained if the assumption is made that lens fiber gap junction channels played a role by closing low resistance pathways locally. The following is a possible scenario: initially osmotic stress caused by polyols spreads uniformly throughout the cortex but because of the tight packing of fiber cells, fiber cell swelling is confined to 'weak spots'. These exist predominantly in a discrete zone (which becomes the liquefaction zone), possibly due to transient structural weaknesses during fiber cell maturation. As fiber cells in this zone swell and rupture their plasma membranes, gap junctions at the interface between ruptured cells and surrounding normal tissue close, thereby spatially limiting the liquefaction process. Naturally, gap junction closure would eventually cut communication between the lens periphery and the core region. The resulting loss of homeostasis could trigger the opacification of the lens core which occurs in the later stages of diabetic cataract.

While this scenario is only a working hypothesis at this stage, it is testable. Dye diffusion and electrophysiological experiments could be designed to monitor low resistance pathways into the lens interior as a function of progressive cataractogenesis. The diabetic rat model allows precise timing of the diabetic condition

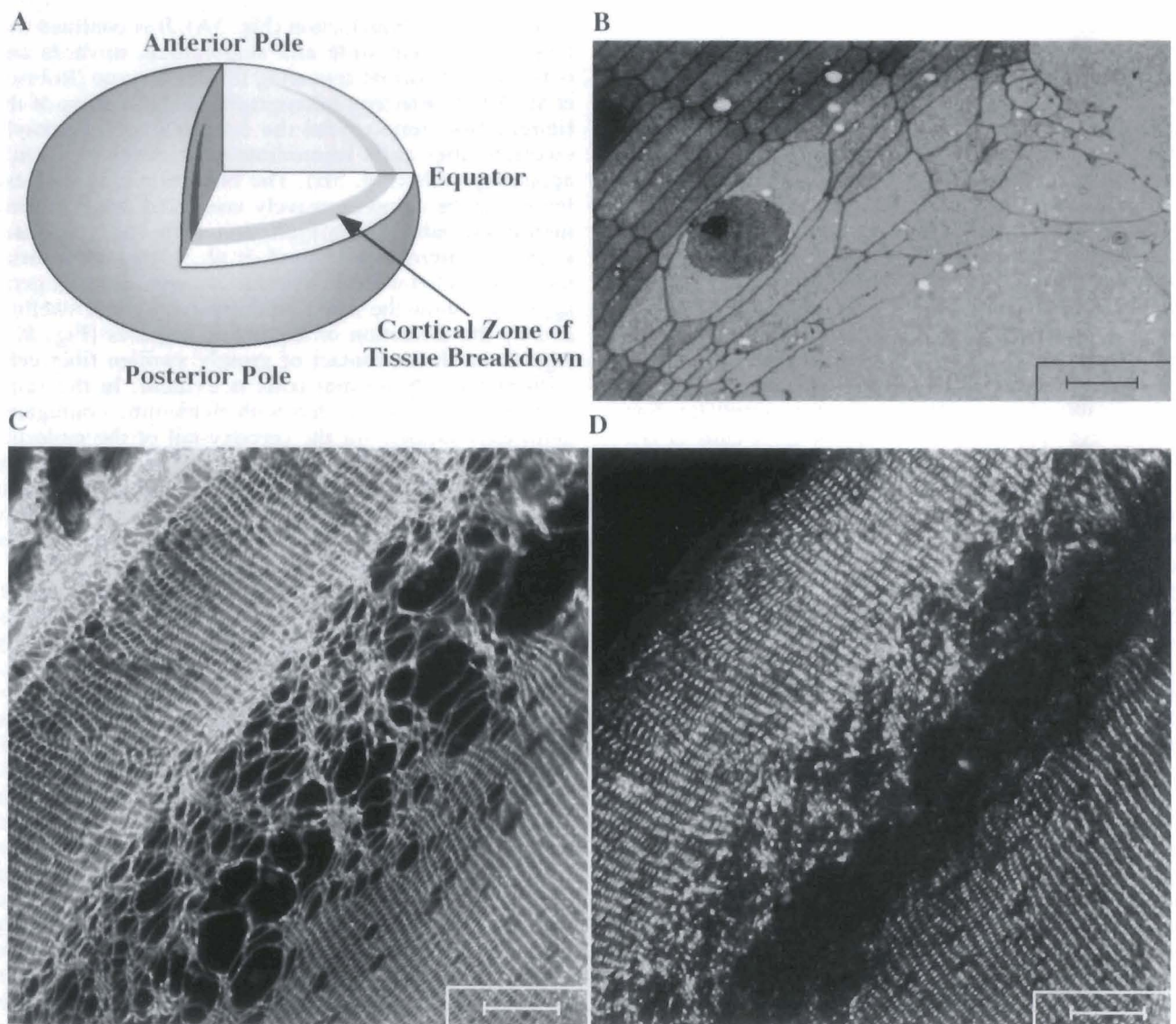


and displays reproducible formation of opacities and, at the histological level, cellular changes leading to tissue disruption.

### Conclusions

The realization that the gap junction channels are comprised of a diverse multigene family has greatly accelerated efforts to define the role intercellular communication plays in controlling and coordinating

many different multicellular events. Knowledge of connexin distribution and expression patterns coupled with studies into the functional consequences of connexin diversity have provided invaluable information on the basic biology of gap junction channels. More recently, additional important knowledge has been gained from studies of disease processes and the role connexins play in these processes. Hence, gap junctional research has become a cyclical process with the implication of a particular connexin in a disease process



**Fig. 5.** Gap junctions and lens cataract. **A.** Schematic drawing of the lens and the cortical zone of tissue breakdown and liquefaction typical for diabetic cataractogenesis. **B.** Fiber cell swelling and membrane rupture in the cortex of a lens taken from a rat fed a galactose diet. This is an equatorial thin section imaged by transmission electron microscopy, and the field of view shows cellular changes at the inner border of the liquefaction zone. Note the transition from normal fiber cells, moderately swollen cells to 'giant' swollen cells (left to right). Multiple membrane ruptures are evident in the middle of the figure. Scale bar: 4  $\mu\text{m}$ . **C and D.** Cortical tissue liquefaction in a lens taken from a diabetic rat 4 weeks after injection with streptozotocin. These equatorial sections were labeled generally for membranes with fluorescein conjugated wheat germ agglutinin (C) and specifically for junctional domains with anti-connexin50 antibodies which were detected with a rhodamine conjugated secondary antibody (D). Note transitions between normal fiber cells, swollen cells of increasing size and large fluid filled spaces within the liquefaction zone and along its borders. Scale bars: 50  $\mu\text{m}$ .



often providing new information on the basic function and regulation of that connexin isoform.

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