# Gap junctions – from cell to molecule

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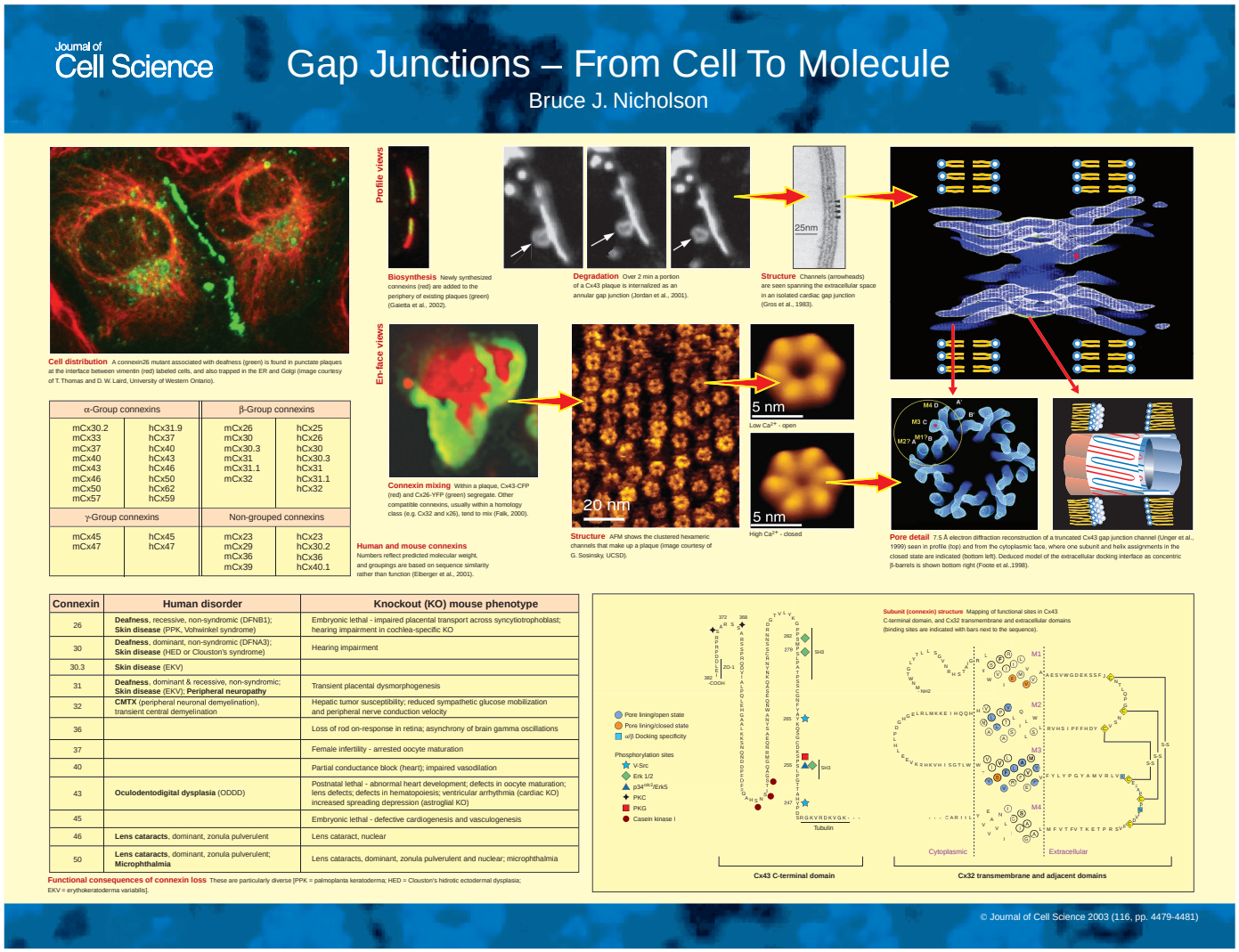
Gap junctions were first defined morphologically as specialized contacts between cells that mediate the direct exchange of small molecules needed to coordinate behavior of multicellular systems. As a functional entity, they have evolved independently three times, if one includes plasmodesmata in plants, which are structurally very distinct from gap junctions but mediate similar functions. The gap junctions of vertebrates and invertebrates are similar in both structure and function, but the former are composed of the connexin family (Eiberger et al., 2001; Willecke et al., 2002), while the latter are composed of members of a topologically similar but unrelated family called the innexins (Phelan and Starrich, 2001). Recently, however, three 'pannexin' genes displaying distant homology to the innexins, but of as yet unknown function, have been found in vertebrates (Panchin et al., 2000).

## Diverse functions

The ubiquitous presence of gap junctions in the metazoa has suggested that they are essential for multicellular life – a conclusion that is supported by the array of different human diseases and mouse phenotypes that arise from defects in these genes. Some of the diseases have a significant impact on the human population, with Cx26 mutations accounting for over 50% of the cases of inherited asymptomatic sensoneural deafness (Kelsell et al., 1997). Among the phenotypic consequences of connexin loss, some appear to reflect the role of gap junctions as ion conductors, such as the loss of retinal rod signaling, cortical asynchrony (Cx36), partial conductance block (Cx40) and ventricular arrhythmias (Cx43) in the heart, and $K^+$ recycling in the ear (Cx26 and Cx30). Others are likely to result from interruption of nutrient transfer between cells, as in the avascular eye lens (Cx46 and Cx50) and placental transfer to the fetus (Cx26 and Cx31). Yet other defects illustrate the probable role of gap junctions in mediating intercellular signaling, such as coordination of neural




| Connexin | Human disorder | Knockout (KO) mouse phenotype |
|---|---|---|
| 26 | **Deafness**, recessive, non-syndromic (DFNB1); **Skin disease** (PPK, Vohwinkel syndrome) | Embryonic lethal - impaired placental transport across syncytiotrophoblast; hearing impairment in cochlea-specific KO |
| 30 | **Deafness**, dominant, non-syndromic (DFNA3); **Skin disease** (HED or Clouston's syndrome) | Hearing impairment |
| 30.3 | **Skin disease** (EKV) | |
| 31 | **Deafness**, dominant & recessive, non-syndromic; **Skin disease** (EKV); **Peripheral neuropathy** | Transient placental dysmorphogenesis |
| 32 | **CMTX** (peripheral neuronal demyelination), transient central demyelination | Hepatic tumor susceptibility; reduced sympathetic glucose mobilization and peripheral nerve conduction velocity |
| 36 | | Loss of rod on-response in retina; asynchrony of brain gamma oscillations |
| 37 | | Female infertility - arrested oocyte maturation |
| 40 | | Partial conductance block (heart); impaired vasodilation |
| 43 | **Oculodentodigital dysplasia** (ODDD) | Postnatal lethal - abnormal heart development; defects in oocyte maturation; lens defects; defects in hematopoiesis; ventricular arrhythmia (cardiac KO) increased spreading depression (astroglial KO) |
| 45 | | Embryonic lethal - defective cardiogenesis and vasculogenesis |
| 46 | **Lens cataracts**, dominant, zonula pulverulent | Lens cataract, nuclear |
| 50 | **Lens cataracts**, dominant, zonula pulverulent; **Microphthalmia** | Lens cataracts, dominant, zonula pulverulent and nuclear; microphthalmia |

*(See poster insert)*

crest migration (Cx43), oocyte maturation (Cx37 and Cx43), myelin development (Cx32 and Cx47), coordination of hepatic sympathetic responses (Cx32) and morphogenesis of the heart, blood, bone, vasculature (Cx43 and Cx45) and skin (Cx26, Cx30 and Cx30.3). A summary of connexin diseases and mouse gene ablation studies appears in Willecke et al. (Willecke et al., 2002).

## Formation and degradation

Gap junctions form punctate 'plaques' at the interfaces between cells, although protein is often seen trapped in the ER and Golgi in some disease-associated mutants. Although not glycosylated, most connexins do pass through the Golgi, and oligomerize late in their biosynthesis (Musil and Goodenough, 1993). There is also some evidence for non-Golgi transport of Cx26 (Martin et al., 2001). Hexameric connexons, or hemichannels, are inserted into the membrane, where they remain in a predominantly closed state (but see below) until recruited to the outside of existing plaques within the membrane (Gaietta et al., 2002). Removal of old connexins can occur through 'budding-off' of whole pieces of the plaque, to form annular gap junction vesicles within one cell (Jordan et al., 2001). Both lysosomal and proteasomal degradation pathways for connexins have been reported (Laing et al., 1998). Surprisingly, connexins themselves are turned over very rapidly, with a half-life of 1-5 hours (reviewed by Saffitz et al., 2000).

## Channel permeability properties

The primary function ascribed to gap junctions has been the transfer of ions and metabolites under about 1000 Da between cells in contact. Although this may seem to be a 'housekeeping' function, several recent studies have revealed a surprising diversity in the permeability properties of gap junctions composed of different connexins. Ion substitution experiments show that the anion/cation preferences of connexins can differ by up to tenfold, while larger permeants (cationic tertiary amines, neutral polyethylene glycols and anionic Alexa fluorophores), show exclusion limits that vary from 7 to 15Å (200 to over 800 in molecular weight), depending on the connexin composition of the channel (reviewed by Harris, 2001). Rates of dye flux through individual channels were 1-3 orders of magnitude higher than those predicted by diffusion, indicating a significant affinity between the dyes and the pore wall (B. J. Nicholson and J. Nitsche, unpublished). This is consistent with findings that different connexins can impart selectivity levels of 10-100-fold for natural permeants such as ATP, AMP, adenosine and glutathione (Goldberg et al., 1999), $IP_3$ (Niessen et al., 2000) and even different cyclic nucleotides (Bevans et al., 1998).

## Heterologous interactions between connexins

The diversity of gap junction channel properties, defined above for channels composed of one connexin, is further increased by the expression of multiple connexin isotypes in most cells. Both heterotypic (different connexins in opposed cells) and heteromeric (different connexins in the same cell) channels have been documented (reviewed by Harris, 2001). Generally, heterotypic channels behave consistent with an in-series combination of homomeric channels, although rectifying channels can be produced (Suchyna et al., 1999). Interactions between connexins seem to be somewhat selective. Heterotypic coupling tends to occur, with some notable exceptions, within an homology group [i.e. $\alpha$ or $\beta$ (Harris, 2001)], dictated by sequences, possibly as few as a couple of residues, in the second extracellular loop (H. Zhu and B. J. Nicholson, unpublished). Within a single plaque, some connexins will segregate, while others mix (Falk, 2000), which suggests that formation of heteromeric channels is also selective, probably within homology classes based on several biochemical studies (reviewed by Harris, 2001). Other than the preferential interactions just described, no common properties link the members of connexin groupings, which have been derived from sequence alignments of the connexins.

## Channel gating and regulation

Gap junction channels are unusual among ion channels in that they typically remain open at rest, and only close under specific circumstances. However, the gating of these channels is among the most complex of any ion channel. They close in response to a number of stimuli that generally reflect the poor health of a cell, including low pH, high intracellular $Ca^{2+}$ concentration, and voltage differences between cells ($V_j$) (reviewed by Harris, 2001). Some channels are sensitive to transmembrane voltage ($V_m$) as well, but this is more common among the invertebrate innexins. Gap junction channels also close in response to a number of physiological stimuli, typically associated with increased cell division. These include growth factors such as PDGF and EGF, oncogenes such as v-Src, and PKC and Cdc2 kinases associated with mitosis that either directly, or indirectly, phosphorylate the C-terminal tail (reviewed by Lampe and Lau, 2000).

## Hemichannels

Prior to incorporation into gap junctions, hemichannels are typically kept closed through a combination of extracellular $Ca^{2+}$ and $V_m$ gates. On docking with a compatible hemichannel in the other cell, intercellular channels are triggered to open ('loop' gating). Some hemichannels (e.g. Cx38, Cx46 and Cx50) can be opened by membrane depolarization and reduced extracellular $Ca^{2+}$ concentration. However, significant circumstantial evidence has implicated open hemichannels of other connexins in physiological processes as varied as extracellularly propagated $Ca^{2+}$ waves (by release of ATP or $NAD^+$), inhibition of apoptosis in osteocytes (both Cx43), and center surround inhibition of cones by horizontal cells (through local extracellular currents from Cx26) (Goodenough and Paul, 2003).

## Molecular structure of the pore

Improved microscopic, biophysical and mutagenic strategies have led to recent advances in our understanding of the structural basis of gap junction function. Atomic force microscopy has provided a dynamic view of conformational changes in the hexameric channels

during gating, but at limited resolution (Muller et al., 2002). However, a major breakthrough in resolving the structure of the pore came with the reconstruction of electron diffraction images of isolated Cx43 gap junctions (Unger et al., 1999). The four transmembrane helices of each subunit can be seen, with one contributing most of the pore lining (red asterisk, top panel of pore detail image). The docking interface between the two hemichannels appears seamless and shows a structure consistent with a previous model deduced from mutagenesis where interdigitation of the two antiparallel β-strand loops of each subunit from opposed hemichannels form concentric β-barrels (Foote et al., 1998).

All connexins have four transmembrane helices, two highly conserved extracellular loops that are held rigidly together by three disulfide linkages and three highly variable cytoplasmic domains. Systematic cysteine scanning mutagenesis of Cx32 has mapped the residues that contribute to the pore lining in open and closed states (Skerrett et al., 2002), which, surprisingly, revealed that the channel is lined with hydrophobic residues predominantly in M3, with some at the cytoplasmic end of M2. Other mutagenesis studies have indicated that M1 and its flanking domains may contribute to the pore lining of hemichannels (Harris, 2001) and are likely to serve as the voltage sensor of the channel (Rubin et al., 1992). The cytoplasmic C-terminal domain serves as a 'ball' to close the channel in response to pH (Morley et al., 1996) and phosphorylation (Zhou et al., 1999), and contains a variety of targets and binding sites for various kinases, scaffolding and signaling proteins that have been most extensively mapped in Cx43. The latter suggest that, like adhesive and tight junctions, gap junctions may be an important nexus for signaling beyond their role in selective exchanges of metabolites between cells.

I apologize to all who have contributed to our understanding of these channels and their functions but who could not be mentioned here because of space constraints. Sincerest thanks go to Jim Stamos who pieced together the collage in the accompanying poster.

## References

**Bevans, C. G., Kordel, M., Rhee, S. K., Harris, A. L.** (1998). Isoform composition of connexin channels determines selectivity among second messengers and uncharged molecules. *J. Biol. Chem.* **273**, 2808-2816.

**Eiberger, J., Degen, J., Roumaldi, A., Deutsch, U., Willecke, K. and Sohl, G.** (2001). Connexin genes in the mouse and human genome. *Cell Commun. Adhes.* **8**, 163-165.

**Falk, M. M.** (2000). Connexin-specific distribution within gap junctions revealed in living cells. *J. Cell Sci.* **113**, 4109-4120.

**Foote, C. I., Zhou, L., Zhu, X. and Nicholson, B. J.** (1998). Pattern of disulfide linkages in the extracellular loop regions of connexin 32: a model of the docking interface of gap junctions. *J. Cell Biol.* **140**, 1187-1197.

**Gaietta, G., Deerinck, T. J., Adams, S. R., Bouwer, J., Tour, O., Laird, D. W., Sosinsky, G. E., Tsien, R. Y. and Ellisman, M. H.** (2002). Multicolor and electron microscopic imaging of connexin trafficking. *Science.* **296**, 503-507.

**Goldberg, G. S., Lampe, P. D. and Nicholson, B. J.** (1999). Selective transfer of endogenous metabolites through gap junctions composed of different connexins *Nat. Cell Biol.* **1**, 457-459.

**Goodenough, D. A. and Paul, D. L.** (2003). Beyond the gap: functions of unpaired connexon hemichannels. *Nat. Rev. Mol. Cell. Biol.* **4**, 1-10.

**Harris, A. L.** (2001). Emerging issues of connexin channels: biophysics fills the gap. *Q. Rev. Biophys.* **34**, 325-472. [Erratum in: *Q. Rev. Biophys.* 35, 109]

**Jordan, K., Chodock, R., Hand, A. R. and Laird, D. W.** (2001). The origin of annular junctions: a mechanism of gap junction internalization. *J. Cell Sci.* **114**, 763-773.

**Kelsell, D. P., Dunlop, J., Stevens, H. P., Lench, N. J., Liang, J. N., Parry, G., Mueller, R. F. and Leigh, I. M.** (1997). Connexin26 mutations in hereditarry non-syndromic sensoneurial deafness. *Nature* **387**, 80-83.

**Laing, J. G., Tadros, P. N., Green, K., Saffitz, J. E. and Beyer, E. C.** (1998). Proteolysis of connexin43-containing gap junctions in normal and heat-stressed cardiac myocytes. *Cardiovasc. Res.* **38**, 711-718.

**Lampe, P. D. and Lau, A. F.** (2000). Regulation of gap junctions by phosphorylation of connexins. *Arch. Biochem. Biophys.* **384**, 205-215,

**Martin, P. E., Blundell, G., Ahmad, S., Errington, R. J. and Evans, W. H.** (2001). Multiple pathways in the trafficking and assembly of connexins 26, 32 and 43 into gap junction intercellular communication channels. *J. Cell Sci.* **114**, 3845-3855.

**Morley, G. E., Taffet, S. M. and Delmar, M.** (1996). Intramolecular interactions mediate pH regulation of connexin43 channels. *Biophys. J.* **70**, 1294-1302.

**Muller, D. J., Hand, G. M., Engel, A. and Sosinsky, G. E.** (2002). Conformational changes in surface structures of isolated connexin 26 gap junctions. *EMBO J.* **21**, 3598-3607

**Musil, L. S. and Goodenough, D. A.** (1993). Multisubunit assembly of an integral plasma membrane channel protein, gap junction connexin43, occurs after exit from the ER. *Cell* **74**, 1065-1077.

**Niessen, H., Harz, H., Bedner, P., Kramer, K. and Willecke, K.** (2000). Selective permeability of different connexin channels to the second messenger inositol 1,4,5-trisphosphate. *J. Cell Sci.* **113**, 1365-1372.

**Panchin, Y., Kelmanson, I., Matz, M., Lukyanov, K., Usman, N. and Lukyanov, S.** (2000). A ubiquitous family of putative gap junction molecules. *Curr. Biol.* **10**, R473-R474.

**Phelan, P. and Starrich, T.** (2001). Innexins get into the gap. *Bioessays* **23**, 388-396.

**Rubin, J. B., Verselis, V. K., Bennett, M. V. L. and Bargiello, T. A.** (1992). A domain substitution procedure and its use to analyze voltage dependence of homotypic gap junctions formed by connexins 26 and 32. *Proc. Natl. Acad. Sci. USA* **89**, 3820-3824.

**Saffitz, J. E., Laing, J. G. and Yamada, K. A.** (2000). Connexin expression and turnover: implications for cardiac excitability. *Circ. Res.* **86**, 723-728.

**Skerrett, I. M., Aronowitz, J. A., Shin, J. H., Kasparek, E., Cymes, G., Cao, F. L. and Nicholson, B. J.** (2002). Identification of amino acid residues lining the pore of the gap junction channel. *J. Cell Biol.* **159**, 349-360.

**Suchyna, T. M., Chiton, M., Nitsche, J., Harris, A. L., Veenstra, R. D. and Nicholson, B. J.** (1999). Different ionic permeabilities for connexin 26 and 32 produce rectifying gap junction channels. *Biophys. J.* **77**, 2968-2987.

**Unger, V. M., Kumar, N. M., Gilula, N. B. and Yeager, M.** (1999). Three-dimensional structure of a recombinant gap junction membrane channel. *Science* **283**, 1176-1180.

**Willecke, K., Elberger, J., Degen, J., Eckardt, D., Romualdi, A., Guldenangel, M., Deutsch, U. and Sohl, G.** (2002). Structural and functional diversity of connexin genes in the mouse and human genome. *Biol. Chem.* **383**, 725-737.

**Zhou, L., Kasperek, E. M. and Nicholson, B. J.** (1999). Dissection of the molecular basis of pp60$^{v\text{-}src}$ induced gating of connexin43 gap junctions. *J. Cell Biol.* **144**, 1033-1045.

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