NEUROSCIENCE

Connexins in disease

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KNOCKOUTS can tell a lot about a gene's function. But they are perplexing if it turns out that loss of function of a seemingly important gene still results in a fairly normal phenotype. Gap-junction researchers are in just this situation now that two groups[1,2] have shown that a relatively benign human disease results from loss-of-function mutations of the connexin 32 gene. This gene encodes a widely expressed connexin or gap-junction-forming protein, with a predicted relative molecular mass of 32K. The disease in question is the X-linked form of Charcot-Marie-Tooth disease (CMTX), a peripheral neuropathy with onset late in childhood. In this disease myelin degenerates, and motor and sensory function are impaired, males being generally more severely affected than females. The big questions raised by the new observation centre on the mechanism by which the myelinating Schwann cells are affected, and why there is no obvious effect in all the other tissues that express connexin 32 (Cx32).

Gap junctions are composed of small aqueous channels that connect the cytoplasmic compartments of the coupled cells[3]. These channels permit small molecules and ions to pass between cells and mediate electrical and metabolic coupling. Twelve connexin isoforms are known in rodents, each encoded by a separate gene[4]. At least four corresponding connexins occur in humans. All are capable of forming homomeric channels. Some connexins are found in many tissues; others in only a few. Single cells can express more than one connexin. Heteromeric hemichannels containing more than one connexin type may occur for some combinations; heterotypic junctions can form between some but not all combinations of homomeric hemichannels. Exploration of the functional differences among the connexins in respect to voltage dependence of junction conductance, permeability, and regulation by phosphorylation and transcriptional controls, is a lively area of research[5].

Schwann cells do in fact express connexin 32 protein, although this was not evident until after the CMTX mutations were identified[1]. Gap junctions were known to form between Schwann cells in culture[6] and *in vivo* during Wallerian degeneration and regeneration[7], but had not been described in intact nerves. Oligodendrocytes, which form central myelin, make gap junctions with each other and with astrocytes, and also form 'reflexive' junctions between cytoplasmic loops in the perinodal regions and Schmitt–Lantermann incisures[8,9]. That there are reflexive gap junctions of this kind in Schwann cells is suggested by immunocytochemical staining for Cx32 at the light microscope level[1]. These junctions could easily have higher conductance and permeability than the cytoplasmic pathway around the myelin spiral, but the importance to the myelin-forming cell is obscure. Whatever gap junctions do for peripheral nerve, their loss is more important distally than proximally, and the effects may not be apparent for years.

Of the 15 different CMTX mutations in the coding region of the Cx32 gene, two are truncation mutants that certainly would lead to loss of function[1,2]. Others are point mutations of highly conserved residues in functionally important regions such as the third membrane-spanning region, which is thought to line the channel, and the extracellular loops which link together in channel formation. A few of the mutations might not cause total loss of coupling, which should be readily testable by expression of the mutant connexins in *Xenopus* oocytes (D. Paul of Harvard University Medical School tells me that he has shown total loss of function in one of the mutants). It will be interesting to see if disease is less severe with mutations that cause partial loss of function. Genetic background may also be important. Two CMTX lineages are wild-type in the Cx32-coding region, and presumably have mutations in upstream regulatory regions.

Connexin 32 is expressed in liver, pancreas, kidney and some neurons[3], as well as in myelin-forming cells. Gap junctions in inexcitable tissues are presumed to allow cells to work together. It now becomes of interest to look more carefully at CMTX subjects for subtle effects on such aspects as liver function, particularly when the organ is challenged by metabolic stress or disease. Gap junctions have also been implicated in transmission of developmentally important signals, because they come and go at appropriate times for them to transmit signals and because interference with coupling may disrupt development[10]. Where the developmentally significant inducing molecules are peptides that act on extracellular receptors, and are too large to cross gap junctions, an inductive role of gap junctional communication can be rejected. In any case, humans do not require Cx32 for essentially normal development.

Gap junctions have also been said to be involved in tumour suppression. In hepatomas, for example, Cx32 is generally reduced in level and intercellular communication is impaired[11]. The hypothesis is that coupling allows growth-suppressing molecules to diffuse into initiated cells from neighbouring normal cells, or permits growth-inducing molecules to diffuse out to the neighbours. More growth allows the accumulation of more genotoxic events, progressing ultimately to malignancy. But the lack of reports of an increase in tumours in CMTX subjects is an indication that Cx32 is not an important tumour suppressor in humans.

In carrier (heterozygous) females, about half the Schwann cells should express the defective Cx32, because of X-chromosome inactivation. The grain of the mosaic of X-inactivation in Schwann

Bacterial designs

BACTERIA have developed spectacular ways of coping with hardship. In times of stress, they may exhibit cooperative survival strategies which cause the colony to aggregate into intricate growth patterns such as that shown here. Similar complex growth patterns are well-known for non-living systems — the electrodeposition of metals, for example. On page 46, Ben-Jacob *et al.* show that the complexity of living systems does not prevent one from being able to develop relatively simple mathematical models of the bacterial growth process using ideas imported from the physical sciences. The key to pattern formation in bacteria, however, is chemotactic communication. Incorporating chemotaxis into the models allows the authors to reproduce the patterns seen experimentally. Philip Ball