Gap junctions: **Fates worse than death?**

Thomas W. White* and Roberto Bruzzone†

The reasons for the molecular heterogeneity of connexin channels *in vivo* remain unclear. Functional replacement of one connexin gene with another has now revealed unexpected phenotypes and shows that cellular homeostasis depends not simply on cell–cell communication but also on the correct types of connexin.

Addresses: *Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115, USA. †Département de Virologie, Institut Pasteur, 75015 Paris, France.
E-mail: twhite@hms.harvard.edu, bruzzone@pasteur.fr

Current Biology 2000, 10:R685–R688

0960-9822/00/$ – see front matter

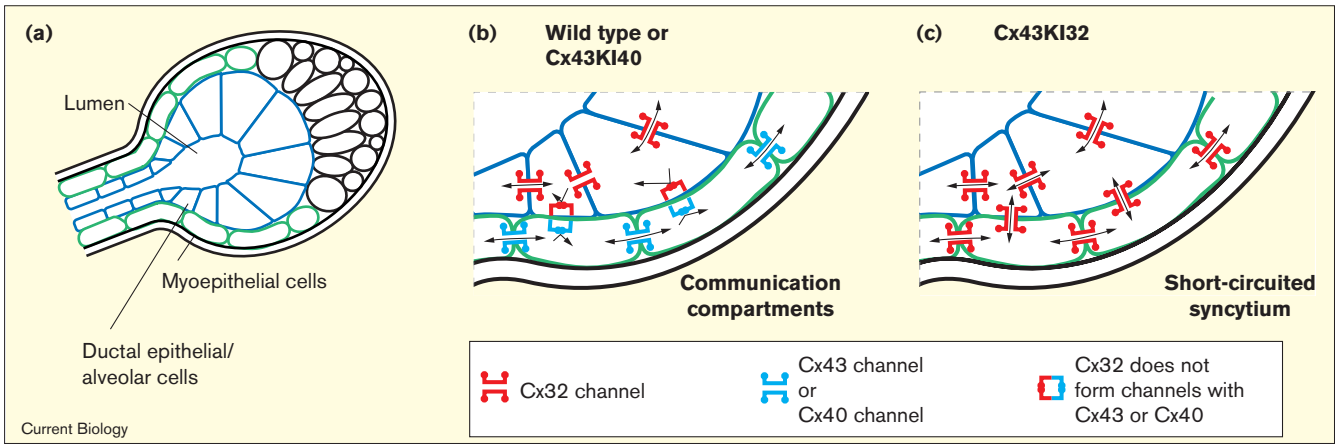
The connexins are encoded by a multigene family and constitute the subunits of gap junction channels, specialized cellular structures providing a pathway for the direct exchange of small molecules and ions between adjacent cells [1]. Critical functions for gap junctions have been elucidated by the discovery of disease-causing mutations in human connexin genes and the observation that mice with targeted deletions of connexins develop distinct phenotypes [2,3]. The descriptions of exquisitely restricted deficits following the impairment of individual connexin genes represents the fruit of many years of research and has led to a profound reappraisal of gap junction function. No longer a passive conduit for the movement of molecules with a molecular mass of less than 1,000 Daltons, intercellular channels are currently considered to provide a selective signaling route whose properties are determined by the molecular identity of the connexins available to the cells in direct communication [4,5].

Several observations, however, remain puzzling. For example, most connexins are expressed in overlapping patterns in many different tissues, yet the phenotypes of gene mutations and ablations are generally restricted [1,2]. This finding suggests either that gap junctional communication is a disposable property in some organs or that functional redundancy occurs — two hypotheses that we do not particularly cherish. In the case of redundancy, one assumes that other connexins would take over the job of the lost gene and the main issue would be simply to keep intercellular communication going. But all connexins are not made equal: in fact they elaborate specific language codes generated on the basis of size and ionic selectivity, rules of compatibility between available connexin partners and distinct gating sensitivity to second messengers and protein kinases [1,6,7]. Thus, if the panoply of connexins expressed at any given time by a group of cells is of importance, one would predict that altering such composition *in vivo* would result in the development of functional abnormalities that demonstrate the stringency of connexin channel requirements in different organs. This is precisely what Plum *et al.* have reported in this issue of *Current Biology* [8], by taking the elegant approach of replacing one connexin gene with another via genetic ‘knock-in’, thereby providing a direct test of the importance of connexin quality versus quantity in intercellular communication.

Starting with the observation that mice lacking the expression of connexin43 (Cx43) die perinatally of cardiac malformation [9], Plum *et al.* [8] asked a simple question: could another connexin replace Cx43 to rescue these mice from death? From sequence data alone, it is difficult to sort the connexin genes into meaningful classes, so the choice of which connexins to exchange with Cx43 was not obvious. The authors opted to replace Cx43 with two other well-studied connexins, Cx32 and Cx40, perhaps because they normally do not interact with Cx43 in functional expression systems [10,11]. The good news is that both lines of knock-in mice were viable; the bad news is that what awaited these mice was a meager future. Not all homozygous animals made it to adulthood, a subset of the pups dying during the first post-partum weeks, and the surviving animals weighed considerably less than their control littermates. More interestingly, two unexpected phenotypes developed that specifically affected the functions of the mammary gland and testis [8].

The basic defect of Cx43-deficient mice, cardiac malformation, was corrected by replacement of Cx43 with either Cx32 or Cx40 (the resulting mice being referred to as Cx43KI32 and Cx43KI40, respectively). Although both connexins were able to restore cardiac morphogenesis to a level compatible with life, the efficacy of their vicarious function was notably different. Thus, Cx43KI32 animals exhibited morphogenic defects similar to those observed in Cx43-deficient mice, albeit significantly less pronounced, whereas the hearts of Cx43KI40 animals appeared to develop normally. It seems premature to speculate on the molecular mechanisms underlying this outcome, although the authors remind us of the differences in both intrinsic and gating properties between Cx32 and Cx40 [4,10,12]. Conductance of single Cx32 channels is lower than that of Cx43 channels, thus resulting in a reduction of coupling strength between two cells joined by the same number of channels. In contrast, the unitary conductance of Cx40 channels is greater and Cx40 may therefore offer a more efficient replacement.

Figure 1



Hypothetical modification of communication compartments in the mammary gland by genetically manipulating the pattern of connexin expression. **(a)** Schematic representation of the mammary gland with ductal and alveolar epithelial cells (blue) surrounded by myoepithelial cells (green). **(b)** In the case of wild-type and Cx43KI40 mice, communication is prevented between alveolar cells expressing Cx32 (red) and myoepithelial cells that synthesize incompatible connexins (Cx43 or Cx40, light blue). **(c)** In Cx43KI32 animals, Cx32 has now replaced Cx43 in myoepithelial cells, leading to the establishment of an illicit communication pathway.

Cx40 is chiefly expressed in the conductive myocardium and it had been hypothesized that its inability to form heterotypic channels with Cx43, which is expressed in working myocardium, would prevent leakage of the electrical stimulus between the two compartments, maintaining a patterned spread of myocardial activation [13]. No signs of an aberrant propagation of ventricular excitation were detected in Cx43KI40 mice, in fact they showed only mild cardiac conduction defects, most notably a susceptibility to spontaneous arrhythmia [8]. These findings, together with the observation that a layer of Cx45-expressing cells prevents the direct interaction of Cx43 and Cx40 [14], suggest that our ideas of communication compartments in the heart need to be revised. It remains to be explained, however, why connexins are expressed in the adult heart according to a cell-specific pattern, given that reshuffling them seems to be without major consequence in animals at rest.

Another intriguing part of the work of Plum *et al.* [8] is the discovery that a new and restricted spectrum of gap-junction-dependent defects appeared in the knock-in mice. The first defect observed was that Cx43KI32 female mice were unable to feed their young, probably due to impaired milk ejection. The epithelial compartment of the normal mammary gland is composed of three cell types, ductal and alveolar epithelial cells that express Cx32, and myoepithelial cells surrounding ducts and alveoli, which normally synthesize Cx43 ([15]; Figure 1a). The myoepithelial cells assist milk ejection by contracting the alveoli during lactation, and the presence of gap junctions between these cells could provide an efficient pathway to synchronize them and improve performance. Because Cx32 is present in the alveolar cells and Cx32 and Cx43 are not competent to form channels with each other [11], alveolar and myoepithelial cells normally form two segregated communication compartments (Figure 1b).

In the Cx43KI32 knock-in mice, Cx32 is now present also in the myoepithelial cells and may illicitly couple them to the alveoli, thereby dissipating signals that are used to coordinate contraction (Figure 1c). If illicit communication is the culprit, one could predict that the Cx43KI40 mice would not suffer this defect, because, like Cx43, Cx40 cannot form channels with Cx32 ([11]; Figure 1b). This indeed seems to be the case as Cx43KI40 mothers can lactate. Studies with fluorescent tracers and/or recordings of electrical coupling will be needed to demonstrate conclusively that avoiding promiscuous coupling with your neighbors may, under certain circumstances, be advantageous.

It was also observed that the male mice in both Cx43KI32 and Cx43KI40 lines were sterile and displayed severely hypotrophic testicles that lacked sperm [8]. Spermatogenesis normally proceeds according to an orderly pattern throughout the reproductive lifespan of the male. Germ cells are interspersed within the supporting Sertoli cells that participate in the nutrition and protection of spermatogonia. It has been shown that spermatogenic cells establish germ-cell- and epithelial-stage-dependent networks of cell–cell communication thought to be important for the initiation and maintenance of spermatogenesis [16].

A very recent study has provided initial evidence that the molecular architecture of junctional communication in the testis may be far more complex than previously anticipated

[17]. RT–PCR analysis identified nine connexin mRNAs in germ cells, eight in Sertoli cells and five in peritubular cells and also showed that the connexin mRNA population varied between seminiferous tubules at different stages. Because gap junctions assemble between Sertoli cells and between Sertoli and spermatogenic cells, it is easy to conceive that multiple, unique routes of cell–cell communication may be established by the assembly of structurally diverse gap junctions that support differences in ion and/or second messenger permeability between cell types. Clearly, the interpretation of the functional significance of such an elaborate communication network remains difficult, as several lines of mice with targeted deletion of connexin genes detected in the testis — for example Cx32, Cx37, Cx40, Cx46 and Cx50 — are fertile [2].

It would have been important to show that the knocked-in genes were well expressed in the testis and their cellular localization was coincident with that of Cx43 (as the authors did for cardiac expression). Assuming that this was the case, one is left with one of three possibilities. First, the substitute connexins are incapable of forming heterotypic channels with other family members, thereby preventing communication between cells normally expressing Cx43 and their neighbours at a critical stage of spermatogenesis. Second, the knocked-in genes led to the establishment of illicit communication between usually segregated cell types, thus bringing us to a situation similar to that postulated above for the mammary gland defect. Third, the range of signaling molecules that can travel through the channels formed by the knocked-in connexins and the gating properties of these channels differ significantly from those of Cx43 channels, thereby pointing to a more subtle defect of cell–cell communication. It seems premature, however, to speculate on the precise nature of the cellular defects that lead to a dramatic arrest of spermatogenesis following the alteration of connexin content in the testis. What this study makes abundantly clear is that spermatogenesis is particularly resistant to the notion of redundancy of connexin function.

Until now, most papers had emphasized the need of communication via connexin channels for the correct maturation of female gonads [18,19], and for contraction of uterine smooth muscle cells at parturition [20], which both express Cx43. Although the knock-in animals do not question the validity of these data, it is interesting to note that follicular maturation seems unaffected by the connexin substitutions. The consequences for pregnancy and delivery are more dramatic in both Cx43KI32 and Cx43KI40 females, but the poor health condition of the underweight and malnourished animals prevents an unambiguous interpretation of the data at this stage. In the case of male reproductive biology, the observations are quite intriguing and raise several questions that will bring a lot of attention to a new field in gap junction biology.

One of the most difficult remaining challenges is to elucidate why connexin gene exchange leads to pathological changes. The study by Plum *et al.* [8] provides some initial clues towards understanding the need for connexin diversity and, like any good paper, actually raises more questions that it can answer. One reason for this is that Cx43 is broadly expressed and its replacement therefore disrupts many different pathways of cell–cell communication, including some that have not yet been studied in detail. The issue of quality versus quantity in connexin physiology could be further tested by taking advantage of the restricted pattern of expression of certain connexins in organs with a simpler architecture. The first example that comes to our mind is the crystalline lens, an avascular organ made of only two cell types that heavily relies on connexin function for cellular homeostasis [21]. In fact, only three connexins are expressed in lens epithelial and fiber cells, and perturbation of cell coupling, either by gene knockout or by mutations in humans, leads to the loss of transparency and formation of cataracts [2,22,23]. While waiting for the results of connexin replacements in the lens, the work by Plum *et al.* [8] elegantly proves that simply continuing to talk is not sufficient — what is needed is the ability to elaborate a more articulate language tailored to suit the needs of different cell types.

References

1. Bruzzone R, White TW, Paul DL: **Connections with connexins: the molecular basis of direct intercellular signaling.** *Eur J Biochem* 1996, **238:**1-27.
2. White TW, Paul DL: **Genetic diseases and gene knockouts reveal diverse connexin functions.** *Annu Rev Physiol* 1999, **61:**283-310.
3. Bennett MVL, Abrams CK: **Hereditary human diseases caused by connexin mutations.** In *Gap Junctions. Molecular Basis of Cell Communication in Health and Disease*. Edited by Peracchia C. San Diego: Academic Press; 2000:423-459.
4. Veenstra RD: **Size and selectivity of gap junction channels formed from different connexins.** *J Bioenerg Biomembr* 1996, **28:**327-337.
5. Bevans CG, Kordel M, Rhee SK, Harris AL: **Isoform composition of connexin channels determines selectivity among second messengers and uncharged molecules.** *J Biol Chem* 1998, **273:**2808-2816.
6. Warn-Cramer BJ, Cottrell GT, Burt JM, Lau AF: **Regulation of connexin-43 gap junctional intercellular communication by mitogen-activated protein kinase.** *J Biol Chem* 1998, **273:**9188-9196.
7. Zhou L, Kasperek EM, Nicholson BJ: **Dissection of the molecular basis of pp60(v-src) induced gating of connexin 43 gap junction channels.** *J Cell Biol* 1999, **144:**1033-1045.
8. Plum A, Hallas G, Magin T, Dombrowski F, Hagendorff A, Schumacher B, Wolpert C, Kim J-S, Lamers WH, Evert M, *et al.*: **Unique and shared functions of different connexins in mice.** *Curr Biol* 2000, **10:**1083-1091.
9. Reaume AG, de Sousa PA, Kulkarni S, Langille BL, Zhu D, Davies TC, Juneja SC, Kidder GM, Rossant J: **Cardiac malformation in neonatal mice lacking connexin43.** *Science* 1995, **267:**1831-1834.
10. Elfgang C, Eckert R, Lichtenberg-Fraté H, Butterweck A, Traub O, Klein RA, Hulser DF, Willecke K: **Specific permeability and selective formation of gap junction channels in connexin-transfected HeLa cells.** *J Cell Biol* 1995, **129:**805-817.
11. White TW, Paul DL, Goodenough DA, Bruzzone R: **Functional analysis of selective interactions among rodent connexins.** *Mol Biol Cell* 1995, **6:**459-470.
12. Bukauskas FF, Elfgang C, Willecke K, Weingart R: **Biophysical properties of gap junction channels formed by mouse connexin40 in induced pairs of transfected human HeLa cells.** *Biophys J* 1995, **68:**2289-2298.

13. Bruzzone R, Haefliger J-A, Gimlich RL, Paul DL: **Connexin40, a component of gap junctions in vascular endothelium, is restricted in its ability to interact with other connexins.** *Mol Biol Cell* 1993, **4:**7-20.
14. Coppen SR, Severs NJ, Gourdie RG: **Connexin45 (α6) expression delineates an extended conduction system in the embryonic and mature rodent heart.** *Dev Genet* 1999, **24:**82-90.
15. Pozzi A, Risek B, Kiang DT, Gilula NB, Kumar NM: **Analysis of multiple gap junction gene products in the rodent and human mammary gland.** *Exp Cell Res* 1995, **220:**212-219.
16. Gilula NB, Fawcett DW, Aoki A: **The Sertoli cell occluding junctions and gap junctions in mature and developing mammalian testis.** *Dev Biol* 1976, **50:**142-168.
17. Risley MS: **Connexin gene expression in the seminiferous tubules of the Sprague-Dawley rat.** *Biol Reprod* 2000, **62:**748-754.
18. Dekel N, Lawrence TS, Gilula NB, Beers WH: **Modulation of cell-to-cell communication in the cumulus-oocyte complex and the regulation of oocyte maturation by LH.** *Dev Biol* 1981, **86:**356-362.
19. Simon AM, Goodenough DA, Li E, Paul DL: **Female infertility in mice lacking connexin37.** *Nature* 1997, **385:**525-529.
20. Garfield RE, Sims SM, Kannan MS, Daniel EE: **Possible role of gap junctions in activation of myometrium during parturition.** *Am J Physiol* 1978, **235:**C168-C179.
21. Goodenough DA: **The crystalline lens. A system networked by gap junctional intercellular communication.** *Semin Cell Biol* 1992, **3:**49-58.
22. Gong X, Li E, Klier G, Huang Q, Wu Y, Lei H, Kumar NM, Horwitz J, Gilula NB: **Disruption of α3 connexin gene leads to proteolysis and cataractogenesis in mice.** *Cell* 1997, **91:**833-843.
23. White TW, Goodenough DA, Paul DL: **Targeted ablation of connexin50 in mice results in microphthalmia and zonular pulverulent cataracts.** *J Cell Biol* 1998, **143:**815-825.