Connexins, pannexins, innexins: novel roles of "hemi-channels"

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Connexins, pannexins, innexins: novel roles of "hemi-channels"

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The advent of multicellular organisms, some 800 million years ago, necessitated the development of mechanisms for cell-to-cell synchronization and for the spread of signals across increasingly large cell populations [168, 185]. Many structures and mechanisms have evolved to achieve such functions [4, 15]. Among these mechanisms, one which is prominent in both the invertebrate and the vertebrate world, across the entire phylogenetic scale, involves the transmembrane flux of large cytosolic and extracellular molecules [4, 15, 65, 66, 69–71, 121, 128, 129, 147, 154, 163]. These fluxes, in turn, are dependent on the formation of specific channels that in all animal classes are made by tetra-span integral membrane proteins [65, 66, 69–71, 121, 128, 129, 147, 154, 163] (Fig. 1).

Three junctional protein families form membrane channels permeable to large molecules

Early electrophysiological and electron microscopy studies converged in the realization that gap junctions, the membrane domains that concentrate intramembrane particles at sites of close membrane apposition, were the physical substrate of cell-to-cell communication in both invertebrate [59] and vertebrate tissues [139] (Fig. 1). The finding that similar drugs (the long-chain alcohols heptanol and octanol) and conditions (intracellular acidification) inhibited intercellular communication in both invertebrate and vertebrate systems [58, 85] was taken as further support that all gap junctions had a similar structure and function. Still, the different size of intramembrane particles, their different partition into the P- and the E-fracture faces of the cell membrane, and the different width of the gap space delineated by the two interacting membranes, suggested that the proteins making vertebrate gap junctions were different from those making the invertebrate structures [96]. These differences have functional consequences, as most elegantly demonstrated by co-culturing cell lines from different animal species. In these experiments, heterotypic coupling was shown between insect cells, as well as between different types of vertebrate cells, whereas virtually no coupling was observed between cells of phylogenetically distant species [48].

There is no longer any question that vertebrate gap junction channels are made by various combinations of different connexin proteins [66, 69–71]. The 20–21 isoforms of this family in rodents and man differ in size, but share a similar membrane topography. Thus, all connexins feature four transmembrane domains connected by two extracellular loops, each comprising three highly conserved Cys residues, a cytoplasmic loop, and both N and C termini in the cytosol (Fig. 2). The difference in size of the different connexin isoforms is essentially due to a different length of the cytoplasmic loop and/or the C terminus [39, 64, 65, 69–71, 109]. Current nomenclature (Table 1) designates connexin proteins as nCxZ, where *n* is the species (e.g.,

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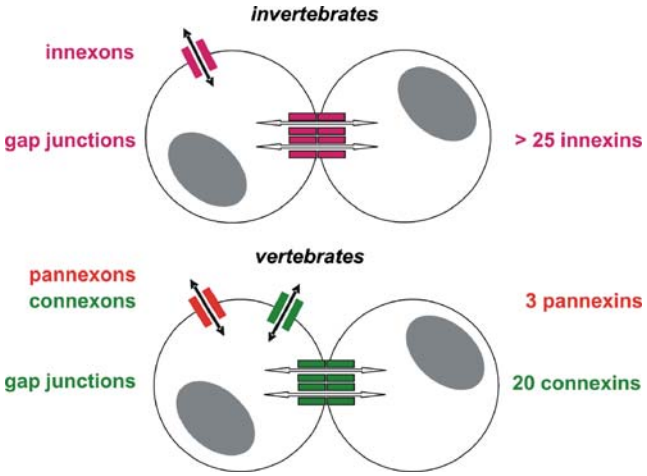

Fig. 1 Innexins, pannexins, and connexins form different types of gap junctional and "hemi-channels." Invertebrates express many different innexins (*purple*; 25 isoforms in *C. elegans*) that may form either gap junction channels for cell-to-cell coupling (*open arrows*) or innexon "hemi-channels" for the permeability of the nonjunctional membrane (*solid arrows*). Vertebrates express three different isoforms of pannexins (*red*) that form pannexon "hemi-channels" and 20 different connexin isoforms (*green*) that essentially form gap junction channels. A few connexin isoforms may also form connexon "hemi-channels"

h, m, r for human, mouse, rat, respectively), and *Z* is the predicted molecular weight, in kDa. The genes encoding connexin proteins are named according to subgroups, in the order of discovery (e.g., *Gjb1* encodes Cx32, *Gjb2* encodes Cx26, *Gja1* encodes Cx43, etc).

Attempts to identify the proteins making invertebrate gap junctions initially revealed the proteins Ogre, Passover, Uncoordinated, and Shaking B in *Drosophila* and *Caenorhabditis* (hence the original OPUS acronym to name these proteins) with no primary sequence homology to connexins [128, 129, 195]. Since that time, more than 25 other junctional proteins revealing significant similarities have been identified in *C. elegans*, and it is clear that many other forms are expressed in other invertebrate species [75, 128, 129, 195] (Table 2). Collectively, these proteins have been named innexins to stress their invertebrate distribution and their role, analogous to that of connexins, in the formation of gap junctions (Fig. 1). Strikingly, innexins also share with connexins a similar structure and membrane topography (Fig. 2), even though the two sets of proteins have no homology in their primary amino acid sequence [75, 128, 129, 195]. Notably, connexins display three conserved Cys within each of the two extracellular loops, whereas innexins only carry two such residues. Thus, it is curious in retrospect that hydra development was reported to be blocked by an antibody prepared against Cx32 [53], that other antibodies have detected connexin-like proteins in anemone [112] and other marine invertebrates [5], and that junctional proteins isolated from hepatopancreas of crayfish and lobster were reported to have a sequence similar to that of rat liver connexins [51].

Sequencing of mammalian genomes has revealed a third family comprising only three genes that code for proteins with a primary sequence showing about 20% similarity to that of innexins [121, 154, 195]. On this basis, these proteins were thought to represent vertebrate homologs of the innexins, and were termed pannexins (Table 3) to encompass both invertebrate and vertebrate members [121, 154]. Like connexins and innexins, all three pannexins display N- and C-terminal domains within the cytoplasm, large extracellular and cytoplasmic loop domains, and four membrane spanning segments (Fig. 2). Like innexins but in contrast to connexins, pannexins contain two Cys residues in each extracellular loop [121, 154]. Furthermore, and in marked contrast with both innexins and connexins, pannexins display consensus sequences for glycosylation [13, 14, 125] (Fig. 2). The distribution of Pnx1 (Pnx1), the most studied form, is widespread and, in most types of cells and tissues, largely overlaps with that of connexins [17, 8]. While at least some phenotypes resulting from loss of specific connexin species are not compensated by pannexin changes [136, 146], suggesting a different function of the two protein families, in other cases, the effects of Pnx1 transfection mimicked that of Cx43, implicating a comparable/overlapping role of these two proteins [93].

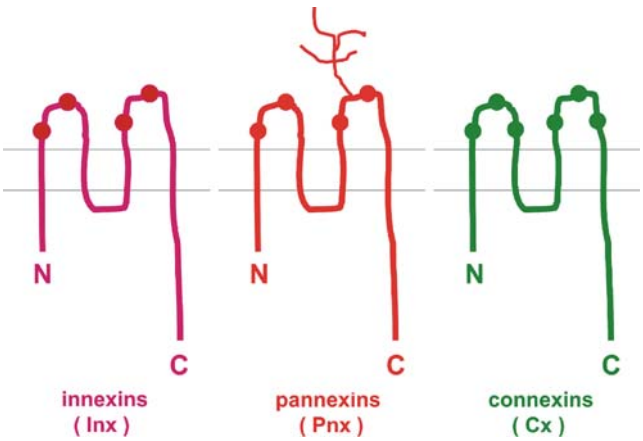

Fig. 2 Membrane topography of the proteins forming gap junctions and "hemi-channels." All invertebrate innexins, vertebrate ortholog pannexins, and nonhomologous vertebrate connexins feature four transmembrane domains connected by two extracellular loops and one cytoplasmic loop, and have both N and C termini in the cytosol. Connexins possess 3 Cys residues (*solid circles*) in each of their extracellular loops. Pannexins and innexins feature only two such Cys residues per loop. Contrasting with both connexins and innexins, pannexin proteins are glycosylated (tree-like structure) on one of their extracellular loop. The *gray areas* schematize the two lipid layers

Table 1 The family of human connexins

Gene	Connexin	Chromosome	Accession number
GJA1	Alpha 1, Cx43	6q22–q23	BC026329
GJA1P1	Alpha 1 pseudogene	5q21.3	M65189
GJA3	Alpha3, Cx46	13q12.11	AF075290
GJA4	Alpha 4, Cx37	1p35.1	M96789
GJA5	Alpha 5, Cx40	6q21.1	121014
GJA6P	Alpha 6 pseudogene	Xp22	100126825
GJA8	Alpha 8, Cx50	1q21.1	U34802
GJAp	Alpha 9, Cx59	1p34	AF179597
GJA10	Alpha 10, Cx62	6q15-q16	AF296766
GJB1	Beta 1, Cx32	Xq13.1	X04325
GJB2	Beta 2, Cx26	13q11–q12	M86849
GJB3	Beta 3, Cx31	1p34	BC012918
GJB4	Beta 4, Cx30.3	1p35–34	127534
GJB5	Beta 5, Cx31.1	1p34.3	BC004379
GJB6	Beta 6, Cx30	13q12	AJ005585
GJB/	Beta 7, Cx25	6q15	AJ414563
GJC1	Gamma 1, Cx45	17q21.31	U03493
GJC2	Gamma 2, Cx47	1q41–q42	AF011643
GJC3	Gamma 3, Cx30.2	7q22.1	AF503615
GJD2	Delta 2, Cx36	15q13.1	AB037509
GJD3	Delta 3, Cx31.9	17q21.1	AF514298
GJD4	Delta 4, Cx40.1	10p11.22	AJ414564
GJE1	Epsilon 1, Cx23	6q24.1	349149

Connexins and innexins form cell-to-cell channels at gap junctional regions of the cell membrane

Expression and deletion studies in a variety of systems have established that connexin hexamers, termed connexons, concentrate at gap junction domains of the cell membrane, where the intercellular space is reduced to a gap 2–3 nm wide. At these sites, the connexons of one cell align with, and strongly bind to the connexons of an adjacent cell, establishing a continuous intercellular hydrophilic pathway (Fig. 1) for the cell-to-cell exchange of multiple types of cytosolic molecules [64, 65, 69–71, 155]. The functional importance of this electrical and metabolic coupling is shown by a variety of striking and tissue-specific phenotypes that can be experimentally induced after overexpression or knock-out of individual connexin isoforms, as well as after the knock-in replacement of one isoform by another [92, 192]. It is further stressed by the identification of a number of diseases that are undoubtedly linked to connexin mutations [43, 57, 90, 94, 127, 143]. A variety of other diseases are thought to be due to altered amounts and/or function of these gap junction proteins [21, 108, 153].

Similarly, innexins oligomerize to form innexons that cluster at gap junctions of invertebrate cells (Fig. 1). Functional expression studies in paired *Xenopus* oocytes demonstrated that several, even though not all innexins also formed intercellular channels [8, 95, 166], and that at least some innexin mutations give rise to phenotypes expected for lack of gap junction-mediated intercellular communication [10, 25, 27, 37, 179]. In fact, it was on the basis of such dysfunctional phenotypes that the OPUS gene family was first identified.

In contrast, and in spite of an initial report [18], pannexons appear unable to form sizable amounts of cell-to-cell channels under most conditions [76, 146, 147, 163] (Fig. 1). This lack of formation of functional gap junctions is likely due to the glycosylation of the extracellular loops of pannexins [13, 14, 125], which, as mentioned above, is not observed for either connexins or innexins. Still, forced expression of pannexins in paired *Xenopus* oocytes increased the conductance of the junctional cell membrane to current carrying ions in a way that can only be accounted for by the formation of pannexin cell-to-cell channels [13].

Connexins, pannexins, and innexins can also form "hemi-channels" in nonjunctional regions of the cell membrane

The finding of nonjunctional permeability induced by expression of connexins, pannexins, and innexins, together with the biochemical, immunological, and functional studies showing that at least some connexon and innexon types may also inserted in nonjunctional regions of the cell membrane [42, 114, 123] challenged the classical views

Table 2 A present view of the growing family of invertebrate innexins

Gene	Innexin	Species	Accession number
LOC100168767	Innexin	*Acyrthosiphon pisum*	100168767
LOC100168642	Innexin		100168642
LOC100162039	Innexin		100162039
LOC100167822	Innexin		100167822
LOC100163262	Innexin-1		100163262
LOC100163012	Innexin-1		100163012
LOC100163664	Innexin 3		100163664
LOC100165879	Innexin 3		100165879
AaeL_AAEL014847	Innexin	*Aedes aegypti*	5565409
AaeL_AAEL014846	Innexin		5565408
AaeL_AAEL014227	Innexin		5579528
AaeL_AAEL011248	Innexin		5574614
AaeL_AAEL008588	Innexin		5570797
AaeL_AAEL006726	Innexin		5568297
AgaP_AGAP001476	AGAP001476-PA	*Anopheles gambiae*	1281698
AgaP_AGAP001477	AGAP001477-PA		1281697
AgaP_AGAP001487	AGAP001487-PA		4577242
AgaP_AGAP001489	AGAP001489-PA		1281682
AgaP_AGAP004510	AGAP004510-PA		1274656
AgaP_AGAP006241	AGAP006241-PA		1276902
LOC725480	Innexin-1	*Apis mellifera*	725480
LOC724832	Innexin-2		724832
LOC55165	Innexin-3		551165
LOC552285	Innexin-7		552285
LOC725553	Innexin shaking B		725553
LOC725513	Innexin shaking B		725513
LOC413472	Innexin shaking B		413472
LOC692687	Innexin 2	*Bombyx mori*	692687
Inx2	Innexin 2		732853
LOC692656	b- iInnexin		692656
Bm1_26040		*Brugia malayi*	6100106
Bm1_40790	Innexin, protein1, a		6103042
Bm1_48735	Innexin, protein1, a		6104632
Bm1_40470	Innexin unc-7		6102078
Bm1_34995	Innexin unc-7		6101888
Bm1_51260	Innexin family protein		6105137
Bm1_48195	Innexin family protein		6104524
Bm1_33440	Innexin family protein		6101576
Bm1_32310	Innexin family protein		6101352
Bm1_31695	Innexin family protein		6101231
Bm1_19285	Innexin family protein		6098762
Bm1_05505	Innexin family protein		6096031
Bm1_35075	Innexin, inx-3		6101904
Bm1_48730	Innexin, inx-5		6104631
Bm1_05870	Innexin, inx-7		6096103
Bm1_51095	Innexin inx-10		6105104
Bm1_25215	Innexin, inx-11		6099945
Bm1_21210	Innexin inx-14		6099146
CBG14811	CBG14811	*Caenorhabditis briggsae AF16*	5640339
CBG14794	CBG14794		56403232
CBG06096	CBG06096		5632895
CBG05256	CBG05256		5641195
CBG22739	GBC22739		5641111
CBG23507	CBG23507		5636295
CBG08617	CBG08617		5636183
CBG10939	CBG10939		5640527

Table 2 (continued)

Gene	Innexin	Species	Accession number
CBG19227	CBG19227		5637409
CBG03987	CBG03987		5627861
CBG17376	CBG17376		5639502
CBG09460	CBG09460		5636929
Inx-1	INneXin	*Caenorhabditis elegans*	180968
Inx-3	INneXin		180866
Inx-4	INneXin		184979
Inx-5	INneXin		181086
Inx-6	INneXin		178231
Inx-7	INneXin		177364
Inx-8	INneXin		178101
Inx-9	INneXin		191694
Inx-10	INneXin		188580
Inx-11	INneXin		179710
Inx-12	INneXin		171944
Inx-13	INneXin		171943
Inx-14	INneXin		172488
Inx-15	INneXin		172004
Inx-16	INneXin		172005
Inx-17	INneXin		172006
Inx-18	INneXin		182801
Inx-19	INneXin		171805
Inx-20	INneXin		188818
Inx-21	INneXin		171930
Inx-22	INneXin		171929
Eat-5	EATing		172480
Unc-7	UNCoordinated		181608
Unc-9	UNCoordinated		181443
CsIV_sQgp2	Innexin-like protein 1	*Campoletis sonorensis ichnovirus ichnovirus*	5075634
CsIV_sQgp1	Innexin-like protein 2		5075635
CpipJ-CPIJ018867	Innexin	*Culex quinquefasciatus*	6052895
CpipJ-CPIJ000259	Innexin		6030978
CpipJ-CPIJ014809	Innexin inx2		6047913
CpipJ-CPIJ012807	Innexin inx3		6045586
CpipJ-CPIJ014745	Innexin inx3		6048414
Dana\GF10895	GF10895	*Drosophila ananassae*	6493762
Dana\GF15974	GF15974		6498775
Dana\GF15976	GF15976		6498707
Dana\GF16006	GF16006		6498807
Dana\GF17986	GF16986		6500765
Dana\GF20329	GF20329		6503039
Dana\GF20330	GF20330		6503040
Dana\GF20357	GF20357		6503065
Dana\GF20443	GF20443		6503150
Dere\GG12050	GG12050	*Drosophila erecta*	655099
Dere\GG14053	CG14053		654448
Dere\GG17574	GG17574		6549828
Dere\GG17644	GG17644		6551473
Dere\GG17645	GG17645		6551556
Dere\GG18003	CG18003		6550369
Dere\GG18075	GG18075		6550410
Dere\GG19626	GG19626		6551474
Dgrij\GH12627	GH12627	*Drosophila grimshawi*	6565092
Dgrij\GH16270	GH16270		6557007
Dgri\GH17771	GH17771		6569440
Dgrij\GH19423	GH19423		6563397

Table 2 (continued)

Gene	Innexin	Species	Accession number
Dgrij\GH24223	GH24223		6565731
Dgrij\GH24273	GH24273		6565708
Dgrij\GH24274	GH24274		6565706
Dgrij\GH24686	GH24686		6565709
Inx2	Innexin 2	*Drosophila melanogaster*	31646
Inx3	Innexin 3		44266
Inx5	Inx5		32901
Inx65	Inx6		31645
Inx7	Innexin 7		33027
Ogre	Optic ganglion reduced		45382
Shak B	CG34358		33062
Zpg	Zero population growth		251414
Dmoj\GI11038	GI11038	*Drosophila mojavensis*	6585922
Dmoj\GI13206	GI13206		6582220
Dmoj\GI15107	GI15107		6584021
Dmoj\GI15723	GI15723		6584424
Dmoj\GI21580	GI21580		6585028
Dmoj\GI21582	GI21582		6585026
Dmoj\GI21669	GI21669		6585029
Dmoj\GI24278	GI24278		6572903
Dper\GL12912	GL12912	*Drosophila persimilis*	6597239
Dper\GL13242	GL13242		6601691
Dper\GL14652	GL14652		6597493
Dper\GL15919	GL15919		6599427
Dper\GL15920	GL15920		6599428
Dper\GL23353	GL23353		6587198
Dper\GL26810	GL26810		6599899
Dper\GL26811	GL26811		6599892
Dper\GL26820	GL26820		6599900
Dpse\GA10092	GA10092	*Drosophila pseudoobscura*	4812514
Dpse\GA13015	GA13015		4801698
Dpse\GA14306	GA14306		4814446
Dpse\GA15552	GA15552		4815318
Dpse\GA18281	GA18281		4814473
Dpse\GA20423	GA20423		4815170
Dsec\GM12279	GN12279	*Drosophila sechellia*	6612617
Dsec\GM13833	GM13833		6611035
Dsec\GM17485	GM17485		6618950
Dsec\GM17487	GM17487		6618948
Dsec\GM17498	GM17498		6618951
Dsec\GM22657	GM22675		6615159
Dsec\GM22683	GM22683		6615104
Dsec\GM22765	GM22765		6614954
Dsim\GDE13120	GD13120	*Drosophila simulans*	6737031
Dsim\GD15600	GD15600		6726412
Dsim\GD16170	GD16170		6725330
Dsim\Gd16171	Gd16171		6725328
Dsim\GD16826	GD16826		6725331
Dsim\GD17854	GD17854		6740252
Dsim\GD18012	GD18012		6729965
Dsim\GD24447	GD24447		6740253
Dsim\GD24658	GD24658		6739865
Dvir\GJ11982	GJ11982	*Drosophila virilis*	6623709
Dvir\GJ15253	GJ15253		6635459
Dvir\GJ15793	GJ15793		6634501
Dvir\GJ15802	GJ15802		6634801

Table 2 (continued)

Gene	Innexin	Species	Accession number
Dvir\GJ15803	GJ15803		6634803
Dvir\GJ15819	GJ15819		6634459
Dvir\GJ18913	GJ18913		6631960
Dvir\GJ23057	GJ23057		6631216
Dwil\GK12587	GL12912	*Drosophila willistoni*	6645049
Dwil\GK13974	GK13974		6650386
Dwil\GK20048	GK20048		6641471
Dwil\GK20049	GK20049		6641472
Dwil\GK25052	GK25052		6648670
Dwil\GK25094	GK25094		6648701
Dwil\GK25295	GK25295		6648282
Dwil\GK25296	GK25296		6648280
Dwil\GK25636	GK25636		6648283
Dyak\GE10490	GE10490	*Drosophila yakuba*	6538133
Dyak\GE15333	GE15333		6526113
Dyak\GE15360	GE15360		6526063
Dyak\GE15472	GE15472		6525881
Dyak\GE15694	GE15694		6525472
Dyak\GE17551	GE17751		6525473
Dyak\GE17553	GE17753		6525476
Dyak\GE20477	GE20477		6533216
HfIV_sB5gp2	Viral innexin-b5.1	*Hyposoter fugitivus ichnovirus*	5076301
HfIV_sB7gp2	Innexin Vnx-b7		5141585
HfIV_sB17gp2	Innexin Vnx-b17		5076289
HfIV_sB10gp1	Viral innexin-bio.1		5076304
HfIV_sC3gp2	Viral innexin-c3.1		5076321
HfIV_sC16gp1	Innexin Vnx-c16		5076283
HfIV_sD5gp1	Innexin Vnx-d5.1		5076286
HfIV_sD5gp1	Innexin Vnx-d5.1		5076286
HfIV_sD5gp2	Innexin Vnx-d5.2		5076285
HfIV_sG1gp1	Viral innexin-g1.1		5076399
HfIV_sG1gp3	Viral innexin-g1.2		5076401
HfIV_sG1gp4	Viral innexin-g1.3		5076402
LOC100118632	Innexin	*Nasonia vitripennis*	100118632
NEMVEDRAFT_vig218755		*Nematostella vectensis*	5502737
LOC656737	Innexin	*Tribolium castaneum*	656737
LOC657243	Innexin		657243
LOC657166	Innexin		657166
LOC661739	b-innexin		661739
LOC656913	Innexin-1		656913
LOC657081	Innexin-7		657081
LOC657000	Innexin-7		657000
Hme1	Innexin	*Hirudo medicinalis*	25264684
Hme2	Innexin		2526687
Hme3	Innexin		77997501
Hme4	Innexin		77997503
Hme5	Innexin		77997505
Hme6	Innexin		77997507
Hme7	Innexin		77997509
Hme8	Innexin		77997511
Hme9	Innexin		77997513
Hme10	Innexin		77997515
Hme11	Innexin		77997517
Hme12	Innexin		77997519
Hvu1	Innexin	*Hydra vulgaris*	86769609
Hvi2	Innexin	*Heliothis virescens*	48926842

Table 2 (continued)

Gene	Innexin	Species	Accession number
Sam1	Innexin	*Shistocerca americana*	10720059
Sam	Innexin		10720060
Sfr1	Innexin	*Spodoptera frugiperda*	37781375
Tci3	Innexin	*Toxoptera citricida*	52630963
Aca1	Innexin	*Aplysia californica*	54398898
Aca2	Innexin		54398902
Aca3	Innexin		54398904
Aca4	Innexin		54398900
Aca5	Innexin		60550112
Aca6	Innexin		60550114
Aca7	Innexin		89118243
Aca8	Innexin		60550118
Cli1	Innexin	*Clione limacina*	8515128
Cli2	Innexin		14210377
Cli3	Innexin		14210379
Cva1	Innexin	*Chaeptorus varipedatus*	15706257
Dja1	Innexin	*Dugesia japonica*	86355173
Dja2	Innexin		86355153
Dja3	Innexin		86355155
Dja4	Innexin		86355157
Dja5	Innexin		86355159
Dja6	Innexin		86355175
Dja7	Innexin		86355161
Dja8	Innexin		86355163
Dja9	Innexin		86355165
Dja10	Innexin		86355167
Dja11	Innexin		86355169

about junctional proteins, by indicating that, at least under certain conditions, some of these proteins may also form functional channels in domains of the cell membrane that are not involved in cell contact. Most strikingly, these channels were found to allow for the leakage of cytosolic molecules, notably ATP and glutamate, into the extracellular medium and to permit the reverse uptake into cells of large extracellular and membrane-impermeant tracers, notably propidium iodide and Lucifer Yellow, [61, 144, 147, 163, 171]. Given that this dye permeability is a major characteristic of connexin and innexin cell-to-cell channels, and that the bidirectional flux of large molecules across the membrane could be abolished by drugs known to block gap junction channels [73, 91, 98, 103, 134], it was proposed that the nonjunctional channels were half of a gap junction channel, made by a nonpaired connexon or innexon (Fig. 1).

Table 3 The family of human pannexins

Gene	Pannexin	Chromosome	Accession number
PNX1	Pnx1	11q21	24145
PNX2	Pnx2	22q13–33	56666
PNX3	Pnx3	11q24.2	116337

The subsequent identification of similar channels in cells (e.g., red blood cells) that do not express connexins or innexins but express pannexins [105], further indicated that these channels may also be made by unpaired pannexons (Fig. 1). For these reasons, these channels were referred to as hemichannels even though this is an obviously semantically improper term to indicate a fully functional and regulated structure [38, 147, 163]. Still, because the term has now gained usual acceptance, we refer to these structures, whether made by single connexons, innexons, or pannexons, as "hemi-channels".

There is now undisputed evidence that Cx46 and Cx50 form such "hemi-channels" when expressed in *Xenopus* oocytes [165] and probably also in the lens [198], and it is widely believed that Cx43 can also form such structures in a variety of cell types [65, 99, 103, 144, 148, 163]. Other reports have proposed that this property is shared by several other connexin isoforms, including Cx23, Cx26, Cx30, Cx30.2, Cx 31.9,Cx32, Cx35, Cx36, Cx37, Cx41.8, Cx45, Cx45.6, Cx55.5, and Cx56 [20, 46, 60, 80, 118, 144, 151, 181, 182, 191]. However, for the reasons that shall be discussed below, it has proven difficult to unambiguously demonstrate the presence of functionally open and unpaired connexons made by these proteins [146, 163]. At least a

few innexins [8, 27, 179] and one (Pnx1) of the three known pannexin proteins [18, 77, 105, 124, 180] also form functionally open innexons and pannexons, respectively (Fig. 1).

"Hemi-channels" formed of different proteins have both similar and distinct biophysical and regulatory characteristics

In keeping with their parallel evolution, similar structure, and membrane topography, most "hemi-channels" share a number of similar biophysical properties that are deemed diagnostic of a composition by one of the three aforementioned families of junctional properties. Thus, they generally feature a large unitary conductance (200–500 pS) [38, 65, 147, 163], although the unitary conductance of certain connexin "hemi-channels" is much lower (e.g., those attributed to either Cx31.9 or Cx36 are <20 pS) [20], a reversal potential near 0 mV when tested with standard internal and external solutions, indicating little selectivity for the major current carrying ions, and a sizable open probability at positive resting potentials (~20% at $V \geq 40$ mV) [38, 65, 147, 163]. Most "hemi-channels" show an in–out permeability to ATP and an out–in permeability to propidium iodide, Lucifer Yellow and 6 carboxyfluorescein [36, 65, 147, 163, 167]. Opening of all types of "hemi-channels" is induced by membrane depolarization ($V \geq 40$–60 mV). Connexin-based "hemi-channels" have been proposed to be activated by low divalent ion concentrations, which for Cx46 and Cx50 shift activation kinetics to lower voltages [164]. Such shift has not been demonstrated for other putative connexin "hemi-channels." Cx43 "hemi-channels" have been reported to be opened under ischemic conditions [11, 33, 34, 84, 99, 135, 151], whereas Pnx1 "hemi-channels" have been demonstrated to be activated by a diverse set of experimental conditions, including mechanical stress, e.g., as provided by an osmotic shock [6, 7, 102, 134], strong depolarizations, and activation of purinergic receptors, including $P2Y_1r$, $P2Y_2r$, and $P2X_7r$, by ATP and other agonists [26, 72, 87, 105, 122, 126, 173, 174, 189] (Fig. 3).

Closure of a number of "hemi-channels" can be induced by several drugs, including carbenoxolone, α-glycyrrhetinic acid, flufenamic acid, mefloquine, and alkanols that also turn off most gap junction channels [14, 124, 169, 177]. The effective concentration of these drugs varies depending on the identity of the channel-forming protein, thereby possibly allowing for discrimination of which channel type is involved in ion or molecular flux. For example, Pnx1 channels appear to be 1,000- to 10,000-fold more sensitive to mefloquine than are Cx43 gap junction channels [36, 79] and less sensitive than connexons to uncoupling flufenamates [16]. Peptides corresponding to the extracellular loops of Pnx1 and Cx43 have also been reported to be effective in blocking both current flow and dye uptake through pannexons and connexons [73, 124, 190]. However, one of the peptides reported to most effectively block Cx32 "hemi-channels" corresponded to an intracellular epitope [40]. Furthermore, the specificity of these peptides has been challenged, as a result both of cross-inhibition of Pnx1 and Cx46 "hemi-channels" in *Xenopus* oocytes, and effective blockade by polyethylene glycols [133]. A more physiological regulation of connexons is seen under conditions of lowered intracellular pH [184, 197] or PKC and MAPK activation [8, 40, 49, 56], which are reported to close most connexin "hemi-channels", and after exposure to either IL1β or TNFα, which is reported to open them [113, 137, 176]. The last finding is quite surprising, given that IL-1 β treatment has been shown in several studies to cause gap junctions to disappear [22, 44, 83, 111, 145]. Conversely, "hemi-channels" may be opened by FGF-1 [149]

The biophysical properties reported for connexin "hemi-channels" differ from those of the gap junction channels formed by the same connexin. Thus, while the unitary conductance of the main state of connexin "hemi-channels" is about twice that of the same connexons in gap junctions, and the substate conductance seen at high positive potentials is generally more than twice that of the corresponding gap junction channel. Voltage sensitivity is also different in "hemi-channels" and gap junction channels. In invertebrates, gap junctions tend to be sensitive to transmembrane (or "inside–outside") potential, closing when either or both cells are depolarized [23, 157], whereas in mammals, gap junctions are closed by the potential across the gap junction (nonzero transjunctional voltage), each connexin having a specific sensitivity, as shown by Boltzmann parameters [65, 161]. Moreover, a fraction of the junctional conductance is usually preserved in gap junction channels even at the highest voltages. This minimal conductance ranges from virtually zero to almost unity for different connexins and represents occupancy of a channel substate. The predominant form of voltage dependence seen in pannexons and connexons "hemi-channels" is conspicuously different from the gating seen in either innexin or connexin gap junctions. Thus, "hemi-channels" open in response to membrane depolarization above about +40 mV, a gating which is attributed to the so-called "loop gate" that is exposed to the transmembrane field in unpaired connexons [65]. For Pnx1, current activation increases with voltage without attenuation, whereas for connexons, there is a decrease in current at the highest voltages, due to the gating to a substate caused by the transjunctional voltage sensor. The gating to substate and its absence in a C-terminally GFP-tagged Cx43, has provided the strongest evidence to date that Cx43 connexons can open in mammalian cells, albeit under nonphysiological conditions [32].

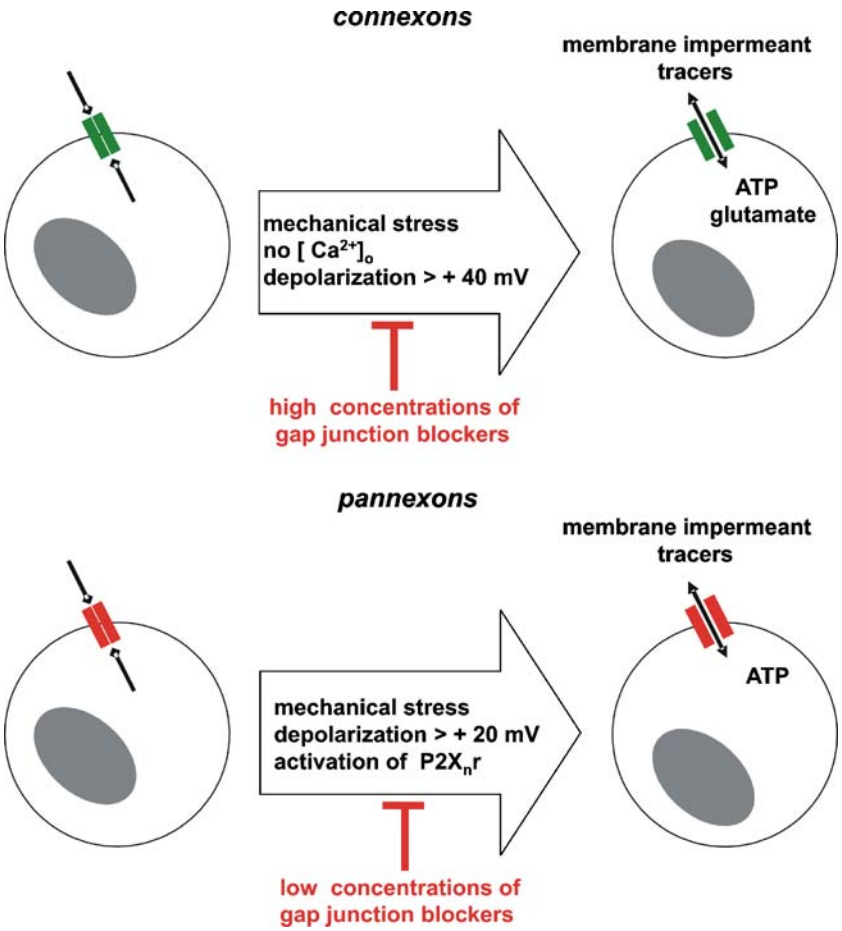

Fig. 3 Conditions leading to the detection of functional "hemi-channels." Functional connexon "hemi-channels" are revealed in the absence of extracellular [Ca^{2+}] or [Mg^{2+}], and upon supraphysiological depolarizations. Pannexon "hemi-channels" are activated in the presence of normal extracellular [Ca^{2+}] and physiologically relevant membrane depolarizations. Many "hemi-channels" of different protein composition are activated by mechanical stress and membrane depolarization

A major difference between pannexons and connexons is that pannexons have been shown to open under physiological conditions, while most connexons have not. Notably, whereas pannexin channels can be opened at normal resting potential and in normal extracellular Ca^{2+} solutions, by mechanical stretch and by P_2 receptor stimulation, connexons have only been demonstrated to open under supra- or pathophysiological conditions (no extracellular Ca^{2+} or Mg^{2+}, depolarization exceeding +40 mV). Although both pannexons and connexons have been reported to be affected by raising intracellular $[Ca^{2+}]_i$ levels, the reality of this regulation is still debated [106].

The identity of the protein forming "hemi-channels" is disputed

Given that innexins, connexins, and pannexins can all form "hemi-channels" which share several common features, including permeability characteristics and sensitivity to blocking drugs, the question arises of which of these protein species actually establishes the functional membrane conductance and permeability. A definitive answer to this question is complicated by the fact that pannexins and connexins have largely overlapping distributions in vertebrates. Also, it is conceivable that, with evolution, different cell types have acquired different types of "hemi-channels" to fulfill a specific role in a specific environment, so that the situation in one cell system might not be applicable to another.

Data from the initial experiments were taken as an indication that "hemi-channels" were made by connexons, mostly because gap junction channels and connexon channels share a conductance for multiple ions, a permeability to large hydrophilic molecules and a sensitivity to the same set of drugs [56, 163, 188]. Furthermore, the conductance of "hemi-channels" was about twice as high as that of gap junction channels made by the same connexin, as would be anticipated if the two connexons joined in series in a gap junction were dissociated from each other.

The most compelling evidence for Cx43 "hemi-channels" comes from HeLa cells overexpressing Cx43, in which currents were evoked by membrane depolarization above +20 mV, single channel conductance was about twice that of Cx43 gap junction channels, conductance was minimally affected by extracellular Ca^{2+} levels, but no channel activity was observed at membrane potentials ≤0 mV [32]. Moreover, the "hemi-channel" formed by Cx43-GFP featured no residual conductance, consistent with properties of gap junction channels formed by this construct, and a N-terminal GFP-Cx43 fusion protein was found not to form "hemi-channels," again consistent with lack of formation of functional gap junction channels by this construct [32].

The major concern regarding the assignment of connexons as functional "hemi-channels" is that the lack of opening of connexons at negative resting potentials is not consistent with the dye uptake observed under such conditions in many cell types [38, 147, 163]. This finding, as well as a variety of expression studies, analysis of cells lacking connexins, and experiments conducted under conditions expected to close gap junction channels have clearly documented that proteins other than connexins can form "hemi-channels" in vertebrate cells [38, 104, 146, 163]. For example, in different cell types, the uptake of extracellular and membrane-impermeant tracers occurs under conditions (presence of extracellular Ca^{2+}, limited depolarization) in which open connexon "hemi-channels" cannot be revealed by electrophysiology [32, 38, 146, 163]. These considerations raise the issue of whether most of the literature published on Cx43 "hemi-channels" is actually reporting other Ca^{2+}-dependent phenomena, such as transport, vesicular uptake and release, or other pathways.

The demonstration that pannexins by themselves form "hemi-channels" was provided by expression of Pnx1 in single oocytes, which lead to the appearance of large currents activating at membrane potentials above −20 mV [16]. These currents were both voltage- and time-dependent, being larger and inactivating more rapidly at larger depolarization. The co-expression of Pnx1 and Pnx2 elicited currents that were larger than those induced by Pnx1 alone, whereas Pnx2 by itself did not yield currents. Because activation was delayed in the co-expression experiments, it was hypothesized that pannexins 1 and 2 also form heteromeric nonjunctional channels [16]. By contrast, Pnx3 neither formed channels itself, nor modified those formed by the other pannexins [16].

It is therefore likely that in a number of cases, the "hemi-channels" attributed to Cx43 were actually due to pannexin activation. One example involves the selection of the J774 macrophage cell line for resistance to ATP-induced cell killing by repeated exposure of the cells to 10 mM ATP. Resistant clones were found to be deficient in Cx43, from which it was inferred that the so-called P2Z receptor or permeabilization pore induced by high concentrations of ATP was the Cx43 "hemi-channel" [12]. This hypothesis was disproved by cloning of the $P2X_7$ receptor [175], by studies showing that the permeabilization pore was present in macrophages lacking Cx43 [3], that J774 cells co-express Cx43 and $P2X_7$ receptors [52], and by experiments in which Cx43 transfection did not induce the permeabilization pore in BHK cells [62]. The demonstration that Pnx1 is found in retinal horizontal cells similarly raises the possibility that the large conductance "hemi-channel" currents recorded from these cells might actually be carried through pannexin channels [45], rather than through connexin "hemi-channels" [42, 155], since the high conductance of pannexons would be expected to provide substantially more ephaptic current at the restricted horizontal-photoreceptor contact than would retinal connexons.

Two independent groups have recently identified Pnx1 as a part of a complex that provides membrane permeabilization to large molecules, following the activation of the $P2X_7$ receptor by ATP or analogues of the endogenous nucleotide [105, 124, 196]. Thus, initial activation of the $P2X_7$r selective cation channels allows for the permeation of molecules up to 900 Da [50, 116]. Two hypotheses have been proposed to account for this change of the ionotropic receptor. The first involves the dilation of the $P2X_7$ cation channel itself [19, 28, 178], while the second involves the recruitment of a protein, most likely Pnx1, forming a lytic pore [81, 150, 175]. Interestingly, the resulting membrane permeabilization, which allows for a nonselective influx of large molecular weight probes [105, 124], does not correlate with the activation of caspase1, nor with the processing and release of IL-1β [124], even though blockade of Pnx1 "hemi-channels" with mimetic peptides prevents both the uptake of the tracer YoPro and caspase1 activation [124]. At any rate, the apparent coupling of Pnx1 activation to that of P2Z/$P2X_7$ receptors, raises the issue of whether pannexin channels can be activated by other receptors. Indeed, the metabotropic $P2Y_1$ and $P2Y_2$ receptors can also activate Pnx1 opening [106]. In addition, uptake of the YoPro tracer can be induced by other P2X receptors, including $P2X_2$ [24] as well as $P2X_4$ and $P2X_5$, presumably via a change of the channel pore [Surprenant, personal communication].

The junctional roles of connexin and innexin channels is unquestionable

A common role of the intercellular channels made by connexins and innexins at gap junctions of vertebrate and invertebrate tissues is to establish direct communication between cells in contact, i.e., to allow for direct and bidirectional exchange of current-carrying ions and other cytosolic, membrane-impermeant molecules [64, 65, 69–71, 109, 158, 192]. Several specific functions have been

experimentally demonstrated to be due to these communications, and many more are attributed to gap junctions on the basis of strong, still circumstantial evidence [109, 158]. The *in vivo* importance of these functions is stressed by the many phenotypes that have been reported after deletion, overexpression, or replacement of specific connexin isoforms in mice [159, 192], as well as by the increasing number of human diseases that have been linked to either connexin mutations or pathogenic single nucleotide polymorphisms [2, 30, 68, 108, 140, 193]. While the role of innexins has been less intensively investigated, the direct intercellular communications mediated by these proteins have been shown to be essential for synapse establishment in the retina and the CNS [158, 159, 192].

More recently, other roles of connexins, that may be independent of the establishment of cell-to-cell channels, have been revealed. Thus, it is now clear that gap junction proteins interact with other membrane and cytosolic proteins and signaling pathways, and may profoundly affect the expression of multiple genes [64, 69–71, 78, 162]. These novel roles call for a reevaluation of the mechanism of some of the previous cell alterations shown to be dependent on gap junction proteins. Whether nonjunctional connexons may also contribute remains to be shown, given that connexin "hemi-channels" can be functionally demonstrated only under supraphysiological or pathological conditions.

The junctional roles of pannexin channels is questionable

There is presently little evidence that pannexins provide direct intercellular communication through gap junctions. Injection of pannexin RNAs indicated that Pnx1, but not Pnx2 and Pnx3, formed gap junctional channels in pairs of *Xenopus* oocytes, that were not very voltage sensitive [16, 18]. Furthermore, the conductance of these channels was about 15% smaller when Pnx1 was co-expressed with Pnx2, which was interpreted as reflecting the formation of heterotypic Pnx1–Pnx2 channels [16, 18]. However, gap junctional communication has so far been reported only after pannexins had been exogenously expressed [18], raising the question of whether the endogenous levels of the proteins, if not their structure and notably their glycosylation, prevent the series assembly of 2 pannexons into a full gap junction channel [13, 14, 125]. Future studies should address this issue and test whether pannexins could, for example, contribute to the synchronization of neurons in the inferior olive and hippocampus, where synchronized oscillations are generated. Indeed, while these oscillations were altered after loss of Cx36, the predominant neuronal connexin, the high frequency oscillations of the neuronal networks was surprisingly unchanged [41, 47, 158], suggesting a compensation by another cell-to-cell communication mechanism. Pannexons, however, seem an unlikely candidate because the large single channel conductance would provide very strong coupling even if only a few channels were functional.

Pannexin "hemi-channels" mediate paracrine cell-to-cell communication

In contrast, several functions dependent on indirect cell-to-cell communication might use large "hemi-channels" for the release of ATP [87], glutamate [194], and epoxyeicosatrienoic acid (EET) [81]. While the mechanism of this release has been repeatedly attributed to Cx43 "hemi-channels," the discovery that pannexons are permeable to ATP under physiological conditions [104] establishes that channels made of pannexins may be involved in such release, that is required for different cell functions.

Calcium waves Two distinct pathways responsible for the inter-cellular propagation of calcium waves have been identified. One involves the diffusion of calcium-mobilizing second messengers (e.g., Ca^{2+}, IP_3, cADP-ribose) from the cytosol of one cell to that of another, through the gap junctional channels made by either connexins or innexins [147, 152]. The other is an extracellular pathway, which involves the diffusion of molecules acting on cell surface membrane receptors [147, 152]. In several cell types, this pathway involves the release of ATP through membrane channels, allowing for the activation of ATP-sensitive purinergic receptors (P2Rs), a family comprised by metabotropic P2Y and ionotropic P2X receptors, on both the very same cell that released ATP (autocrine pathway) and/or the neighboring cells (paracrine pathway) [147]. The latter, extracellular pathway operates in many cell systems [47, 67, 119] and, at least in some of these cases, is dependent on ATP release and on the interaction of the nucleotide with membrane receptors [62]. Many mechanisms could account for the release of cytosolic ATP across the cell membrane, including several types of channels (volume-activated anion channels, VDAC, pore forming $P2X_7r$). Previous studies concluded that Cx43 "hemi-channels" were instrumental to this end between cultured astrocytes exposed to low divalent cation solutions, because drugs blocking gap junctions prevented the intercellular propagation and amplification of the calcium waves [35, 170, 172]. However, further studies showing that amplification of intercellular calcium waves was still present in cultures of astrocytes from Cx43-null mice, and absent in those from $P2X_7r$-null mice [173], have since demonstrated that the pore-forming $P2X_7$ receptor is the most likely candidate to mediate ATP release. More recently, Pnx1 has been shown to be part of the $P2X_7r$

complex, providing both a site for ATP release [104, 105], and a mechanism for amplifying the extent to which intercellular calcium waves spread between astrocytes [147].

Vasodilation It has long been known that erythrocytes release ATP following shear stress and hypoxia [11, 160]. However, it is only recently that the mechanism involved in such release has been shown to depend on Pnx1 "hemi-channels" [104]. Thus, erythrocytes express channels with a unitary conductance (400–500 pS), voltage dependence and mechano-sensitivity of "hemi-channels," and express Pnx1, but no connexins. Pnx1 "hemi-channels" were shown to be permeable to ATP and to large molecular weight dyes, such as carboxyfluorescein. ATP release from erythrocytes was potentiated by high K^+ and osmotic shock, and prevented by carbenoxolone at concentrations lower than those usually required to block connexin gap junction channels. The physiological contribution of Pnx1 was proposed to be related to the local control of blood flow, whereby the ATP released from oxygen-deprived or shear-stressed red blood cells would stimulate purinergic receptors on nearby endothelial cells, initiating the intercellular propagation of a calcium wave. In turn, the elevation of intracellular calcium would induce the release of NO onto the vascular smooth muscle, leading to vasodilation and to a consequent increase in perfusion. The ATP released from erythrocytes could also feedback activating the $P2X_7$ receptors of these same cells, that normally mediate the release of EET through Pnx1 and CFTR channels, given that both carbenoxolone and the CFTR inhibitors glibenclamide and niflumic acid prevented basal and ATP-evoked release of EET [81]. Since EET is an important player in the regulation of vasomotion in several vessels [66], pannexons stand out as crucial channels in this modulation.

Taste sensation Gustatory receptor cells do not display the exocytic machinery involved in neurotransmitter release of most other neuronal cells, and hence cannot release ATP molecules via a vesicular-dependent pathway, yet they are able to convey information about the quality of tastants, via afferent fibers. The taste buds involved in the perception of sweetness and bitterness contains sensory neurons equipped with G-coupled receptors which are involved in the transduction of information, while the buds involved in the perception of saltiness and sourness transduce the information through ion channels [110]. Recent studies have indicated that ATP and serotonin (5-HT) are key mediators in the transduction mechanism of sweet–bitter taste buds, via the ATP release that takes place after tastant presentation. In the extracellular medium, ATP stimulates P2 receptors of the presynaptic taste neurons, leading to the release of 5-HT from these cells. A previous report had implicated Cx43 "hemi-channels" in this mechanism, mostly because conditions known to block gap junction channels, also blocked the outward ATP currents, and in spite of the fact that other conditions acting on gap junctions (e.g., niflumic acid, carbenoxolone, quinine) had no effect on the ATP flux [142]. Blocking conditions included the use of connexin mimetic peptides, which have since been shown to block more efficiently Pnx1 "hemi-channels" than connexin "hemi-channels" [190]. A recent study now indicates that the ATP release takes place through Pnx1 channels of receptor neurons [77]. Furthermore, immunocytochemistry shows that Cx43 is not expressed in receptor cells but in the nearby epithelial cells of taste buds [77]. Thus, it is likely that pannexons are the privileged route for the ATP release which modulates taste sensation.

Pannexin "hemi-channels" mediate intracellular signaling

There is also increasing, still circumstantial evidence for several intracellular functions of pannexons.

Immune response Pnx1 was recently proposed to be involved in the immune response, by associating with the $P2X_7$ receptor signaling cascade to form the inflammosome [88, 124]. This complex of cytosolic proteins induces the activation of caspase1, which is necessary for the processing of interleukin IL-1β and IL-18, two important components of the inflammatory response. Activation of $P2X_7$ receptors by ATP is essential for IL-1β release [50, 86, 126], and appears to be dependent on functional Pnx1 "hemi-channels" [105, 124]. Pnx1 is also necessary for the Toll-like receptor-independent formation of inflammosomes comprising cryopirin [88] which, in turn, is required for the activation of caspase1. It has also been suggested that Pnx1 may trigger the activation of the cryopyrin inflammosome, by mediating the passage of bacterial products from endosomes into the cytosol [88].

Ischemia and cell death Oxygen and glucose deprivation (OGD) induces neuronal necrosis due to imbalance of intracellular ionic concentrations [63, 99, 101, 199]. Large 500 pS conductance events characteristic of Pnx1 "hemi-channels" but not of the channels that could potentially be made by co-expressed connexins, was activated in freshly isolated mouse hippocampal pyramidal neurons following OGD [180]. The OGD-induced currents were blocked by carbenoxolone and La^{3+}, but not by the rat $P2X_7$ receptor antagonist brilliant blue G. Moreover, after microinjection of calcein green into hipocampal neurons in situ, the efflux of the large fluorescein tracer was only observed following OGD, which was also prevented by carbenoxolone [180]. Although the study does not demonstrate the mechanism

whereby OGD induced Pnx1 activity, it was suggested that pannexons contributed to the anoxic depolarization, a frequently observed phenomenon in ischemic conditions, which results in neuronal death [180]. Recently, Pnx1 has also been implicated in the leakage of calcium from the endoplasmic reticulum [187], which is another event promoting neuronal necrosis following CNS ischemia [180].

The co-expression of Pnx1 and $P2X_7$ receptors induced zeiosis of *Xenopus* oocytes following activation of the receptor by extracellular ATP, a phenomenon which was not observed when oocytes were injected either with Pnx1 mRNA alone or with both Pnx1 and the metabotropic purinergic P2Y receptor [105, 106]. These observations suggest that although activation of P2 receptors can induce Pnx1 currents, it is the specific signaling through the $P2X_7$ receptor that leads to cell death.

Recent evidence shows that conditions of oxidative stress, which are expected to favor apoptosis in different cell systems, result in the loss of "hemi-channel" currents, variably attributed to connexons or pannexons [88, 124, 135, 138, 152, 156, 169].

Growth control and tumorigenesis Several studies have provided evidence that the degree of cell coupling as well as the level of connexin expression are reduced in many types of tumor cells and that there is a negative correlation between tumor grade and connexin function and/or expression [89, 97, 131]. Because forced expression of connexins in transformed cells reduces the neoplastic phenotype, it has been proposed that connexins display a tumor suppressor role, by a mechanism likely to be independent of cell coupling and rather related to altered cellular distribution of connexin-binding partners that display transcriptional activity, such as Src, β-catenin and NOV [1, 54, 55, 89, 100 120], and/or to changes in the expression of multiple connexin-dependent genes [29, 78, 97, 115, 162]. Whether and how some of these mechanisms are also dependent on nonjunctional connexons remains to be fully established [141]. Still, clones of glioma C6 cells stably expressing either a myc- or eGFP-tagged Pnx1 had a reduced proliferation and motility compared to cells expressing only eGFP, suggesting a tumor-suppressive role of pannexons [93]. This role was confirmed *in vivo*, inasmuch as mice injected with Pnx1-expressing C6 cells developed smaller tumors than mice injected with eGFP-expressing C6 cells [93]. Because dye coupling mediated by Cx43 was increased and cell morphology altered in the Pnx1-transfected glioma cells, it was proposed that the tumor suppression action of Pnx1 involved both gap junctional and non gap junctional mediated mechanisms [93].

Calcium homeostasis Control of the steady-state levels of cytosolic calcium involves a dynamic equilibrium between the active uptake of the cation into the endoplasmic reticulum (ER), which is mediated by the ATP-dependent Ca^{2+} pumps of the SERCA family, and the passive diffusion of Ca^{2+} from the ER into the cytosol. Several candidates have been proposed to account for the latter passive diffusion, including the reversal of SERCA, translocon channels, IP_3 or ryanodine receptors, Bcl2, Pnx1 and Ca^{2+} ionophore-like channels [31, 107, 117, 130, 183, 186, 187, 200]. Interestingly, transfection of eGFP-Pnx1 in two cell lines expressing endogenous Pnx1 transcripts lead to the accumulation of the tagged-Pnx1 in the ER, which was associated with a reduced thapsigargin-induced Ca^{2+} release and with a higher Ca^{2+} efflux [187]. Conversely, downregulation of endogenous Pnx1 by siRNA strategy, decreased the efflux rate of Ca^{2+} from the ER [187]. Although these results are consistent with the idea that Pnx1 may provide leak channels and therefore contribute to intracellular calcium homeostasis, several open questions still remain. For instance, it would be expected that Pnx1 be tightly regulated by ER Ca^{2+} levels, such that Pnx1 channels would open when these levels were high and close when these levels decrease. Such regulation seems unlikely given that the calcium concentration is much higher in the ER (mM range) than in the cytosol (nM range), and that pannexons are opened by micromolar Ca^{2+} concentrations [106]. At any rate, the Pnx1-mediated leakage of ER Ca^{2+} could represent a key event in

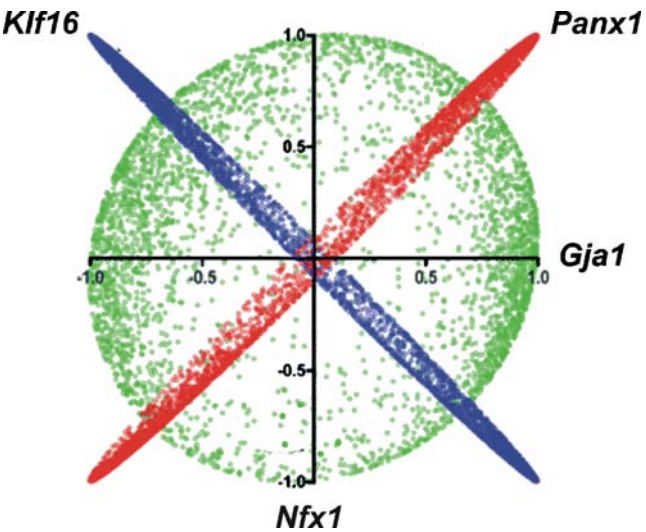

Fig. 4 Cx43 and Pnx1 control the expression of a similar set of genes. Log–log plots of Pearson's coefficients for *Gja1* (X axis), *Panx1*, *Klf16*, and *Nfx1* (Y axis) with all other genes. The red plot indicates that almost all genes are similarly coordinated with that of the two junctional proteins (overlap of the coordination profiles—OVL=93.2). By contrast, the *blue plot* shows that almost all genes exhibit opposite coordination for *Gja1* and *Klf16* (Kruppel-like factor 16; OVL=−94.7). Moreover, the *green plot* indicates that for other genes there is no association of coordinated expression between *Gja1* and the other gene (here, *Nfx1*, nuclear transcription factor X-box binding 1; OVL=−0.4)

triggering the aforementioned neuronal necrosis following CNS ischemia [187] and tumor suppression [93].

"Hemi-channel" proteins modulate gene expression

Microarray analysis of the transcriptome has revealed that the expression of a wide variety of genes is significantly perturbed after loss of individual connexins [29, 78, 115]. Most of these changes can be to a large degree predicted assuming that in the wild-type tissues, the expression of a connexin gene is functionally linked to that of multiple other genes [78]. Thus, when the coordination profiles of individual genes are compared, the striking likeness of certain gene pairs contrasts with the dissimilarity of others. This approach has revealed that Cx43 and Pnx1 expression is very similarly interlinked to that of all other genes (Fig. 4), over the entire range of coordination values. This striking similarity predicts that an up- or a downregulation of either the Cx43 or the Pnx1 gene would equally affect the cell transcriptome, thus probably resulting in a similar phenotype. The data further suggests that, at the transcriptome level, the overexpression of the Cx43 gene may compensate for the underexpression of the Pnx1 gene, and vice versa. However, this compensation could not be expected to correct those phenotypes resulting from altered direct cell-to-cell communication, given the quite limited efficacy of pannexons to form functional gap junction channels.

The present and the future

The discovery that connexin, pannexin, and innexin proteins may form "hemi-channels" in nonjunctional regions of the cell membrane, in addition to paired innexons and connexons forming the classical cell-to-cell channels at gap junctions, has opened a new field in the area of junctional proteins. First, these findings provide a clear view of some of the mechanisms that mediate paracrine intercellular signaling. Second, they provide innovative insights into the regulation of several other cell functions, for which the occurrence of "hemi-channels" provides a plausible if, in most cases, still putative mechanism. Future work should now expose these novel ideas to direct experimental testing. Even if many of the current speculations we have now about "hemi-channels" would turn out to be erroneous concepts, we are certainly entering a time of exciting, new love romance with junctional proteins [132; Bennett MV, talk at the last International Gap Junction Conference, Helsingør, Denmark, August 2007].

Romance, should not detract from the need of a rigorous and critical assessment of the data, notably with regard to the nature of the proteins making "hemi-channels" in different tissues. As repeatedly stressed in this review, this is still an area of hot debate. Many other questions remain to be addressed. For example, can pannexins form gap junction channels in vivo, e.g., under conditions preventing the glycosylation of their extracellular loops? What is the function, if any, of Pnx2 and Pnx3? Are pannexins also forming functional "hemi-channels" in the membranes of cell organelles? What are their physiological functions *in vivo* ? Do they play any role in the pathogenic mechanisms that cause human diseases ? If so, can we act on "hemi-channels" to correct these dysfunctions ? A nonambiguous answer to most of these questions implies that we have the experimental tools (drugs, antibodies, peptides, siRNAs, knockout pannexin models, and transgenic animals over-expressing selected pannexins in a cell specific manner) to specifically interfere with the levels and/or the function of selected types of "hemi-channels." It also requires that cell and animal models lacking or overexpressing "hemi-channels" be available for a direct testing of their function. Normal tissues and cells spontaneously lacking detectable "hemi-channel" function, in spite of expression of Cx36, Pnx1, and the $P2X_7$ receptor, have been recently reported [146], and a mouse null for Pnx1 is said to be viable, possibly without any obvious phenotype (H. Monyer, R. Bruzzone, personal communication). These models should be helpful in approaching some of the many open questions raised by the physiologically paradoxical plasmalemmal "hemi-channels".

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