Cell. Mol. Life Sci. 65 (2008) 376 – 394
1420-682X/08/030376-19
DOI 10.1007/s00018-007-7200-1

Cellular and Molecular Life Sciences

Review

Pannexins and gap junction protein diversity

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Received 22 April 2007; received after revision 9 September 2007; accepted 19 September 2007
Online First 5 November 2007

Abstract. Gap junctions (GJs) are composed of proteins that form a channel connecting the cytoplasm of adjacent cells. Connexins were initially considered to be the only proteins capable of GJ formation. Another family of GJ proteins (innexins) were first found in invertebrates and were proposed to be renamed pannexins after their orthologs were discovered in vertebrates. The lack of both connexins and pannexins in the genomes of some metazoans suggests that other, still undiscovered GJ proteins exist. In vertebrates, connexins and pannexins co-exist. Here we discuss whether vertebrate pannexins have a non-redundant role in animal physiology. Pannexin channels appear to be suited for ATP and calcium signaling and play a role in the maintenance of calcium homeostasis by mechanisms implicating both GJ and non-junctional function. Suggested roles in the ischemic death of neurons, schizophrenia, inflammation and tumor suppression have drawn much attention to exploring the molecular properties and cellular functions of pannexins.

Keywords. Gap junctions, cell-cell communication, pannexin, innexin, connexin, hemichannel, calcium wave.

Introduction

Cell-cell communication across plasma membranes separating individual cells is critically important for development, normal function and pathological processes in multi-cellular organisms. A variety of mechanisms have evolved to support such communication, including plasmodesmata in plants, septal pores in fungi and gap junctions (GJ) in animals. Some specialized membrane structures, like fusion pores and communicating microtubes, add to the list of ancient communication mechanisms which crossed the borders of different living kingdoms [1-5].

GJs stand apart in this list as evolutionarily conserved, but functionally advanced proteinaceous channel structures with regulated gating that support bidirectional transport of ions, small molecules and metabolites across membranes [6-13]. GJ-mediated communication creates an ionic and metabolic syncytium in the compartments of interacting cells and allows their functional synchronization. GJs are composed of membrane proteins that form coaxial cell-cell junctional channels gated by pH, divalent cations, transmembrane potential, phosphorylation, etc. [14,15]. In pioneering works in the 60s and 80s experimental data for GJ function was collected from numerous bio-

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logical models both in vertebrate and invertebrate species and in various tissue and cell types. It has been concluded that gap junctions a) are a universal evolutionary tool of intercellular communication, as they serve similar functions in all multicellular animals (Metazoa); b) provide a universal route for intercellular communication in various tissue and cell types of the same organism. They are ubiquitous and the most common type of intercellular communication. When connexins were identified as the molecular components of vertebrate GJs about 30 years ago [16] and their sequences were determined [17], it appeared that the situation was clear and simple: one protein family to serve one function forming GJ channels connecting the cytoplasm of two adjacent cells. This ideal picture looks much more complicated now, as it has become clear that there is more than one family of proteins that form GJs. In this review, we will briefly discuss how many different GJ protein families exist in vertebrates and other phyla, and how they are interrelated in conjunction with the recent discovery of the pannexin family of junctional proteins. We will focus primarily on mammalian pannexins and compare them to other GJ proteins.

Pannexins obtained their name for their presumptive ubiquitous presence in multicellular animals, *Metazoans* (in contrast to *chordate*-specific connexins) [18,19]. Yet here we suggest that some *Metazoans* that do not have either connexins or pannexins may use other, still unidentified proteins to make GJs. On the other hand, we discuss more, newly revealed functions for pannexins, suggesting that these proteins play a broad role in the physiology of cells and tissues. Until recently, the ability of two apposing half-channels ('hemichannels') to couple together with their counterparts and form intercellular channels was considered the fundamental structural characteristic of GJ proteins [20]. Yet it became obvious that, apart from their GJ function, unpaired hemichannels made of connexins or pannexins (called connexons or pannexons, respectively [12,21,22]) may act in single-cell membranes similarly to regular ion channels. The capability of the pannexin channel for ATP-induced ATP release, their opening on elevation of intracellular Ca^{2+} and their insensitivity to physiological (1–2 mM) levels of extracellular Ca^{2+} allowed researchers to suggest that pannexins may play a key role in long-range Ca^{2+} wave propagation, vasodilation, initiation of inflammatory response [23-25] and ischemic death of neurons [26]. A new prospect for pannexin-mediated function in vertebrates was recently highlighted by demonstrating a potential role of human pannexin1 (PANX1) protein in tumor suppression [27].

Historical overview

Establishment of the low-conductance electrical coupling between contacting cells was the basis of discovery of connexins [28]. This capacity, currently described for connexin and pannexin/innexin protein families, allows establishment of electrically tight molecular channels crossing the lipid bilayer that separates neighboring cells. The ability of GJ channels to pass small molecules and metabolites in addition to ions served to further expand the function of GJs [11,29-31]. Significantly, different connexin and pannexin/innexin family members can produce channels with distinct unitary conductances, molecular permeability, and electric and chemical gating sensitivities. Combined with very selective expression patterns [32-35], such specificity allowed for connecting various cell populations into distinct, electrically and metabolically coupled networks. Electrically coupled astrocyte and neuronal networks in the brain and retina [36-38], metabolically and osmotically coupled lens cell syncytium [31,39], developmentally significant coordination between myocytes [40,41], and between oocytes and granulosa cells represent a few examples of such networks [42,43]. The connexin GJ channels were shown to be homomeric or heteromeric, based on the subunit composition; homo- and heterotypic, where pairing connexons are composed of different subfamilies. The ability of specific connexin subfamilies to pair largely determines heterotypic specificity, and whether the two neighboring cells are able to communicate [44-46].

Hemichannels formed by some connexins can be recruited as regulated pores in non-junctional membrane [14,45,47-49]. These properties further diversify the functionality of GJ proteins to support communication not only between the cytoplasm of neighboring cells but also between the cell and the extracellular media, and to regulate cell volume [21,50,51]. Hemichannel activity of GJ proteins is routinely detected using functional assays like permeability to dyes, ions, conductance between cytoplasm and extracellular media, and functional blocking by pore inhibitors and mimetic peptides [52,53]. These features are characteristic of some connexins, vertebrate pannexins and, most likely, will be confirmed for some invertebrate pannexins/innexins [54,55].

The discovery of an 'alternative' family of GJ proteins coexisting with connexins in vertebrates stirred up an interest in pannexins [18,19,56,57], and suggested that much of the pharmacological data previously attributed exclusively to connexin function should be reconsidered [55,58,59]. Combined with the ubiquitous expression of Panx1, the unique properties of the channels they form and distinct mechanisms of

regulation make pannexins the emerging star of secondary messenger signaling. The mechanosensitivity, permeability to ATP and lack of gating by external Ca^{2+} suggest that pannexins, rather then connexins, might represent the channel more suited for the control of intracellular Ca^{2+} homeostasis and intercellular Ca^{2+} signaling [25,57,59-62]. In contrast to connexin channels, which become coupled very soon after incorporation into the membrane [63], the available functional data on vertebrate pannexins present abundant evidence of their active function in non-junctional membranes [59,60,64]. For more detailed features of invertebrate pannexins/innexins see recent reviews [65-67]. Significantly, a variety of external and internal stimuli, of both physiological and pathological nature, were shown to trigger the opening of the pannexin channel, the non-selective pore characterized by large currents and low molecular weight (< 900 Da) dye uptake [57,59-62]. However, determining of the exact functional roles for pannexons and how they differ from connexons is undermined by lack of specific channel blockers, pannexin gene knockout animals, and ultrastructural data on the localization and membrane organization of pannexin channels.

Evolution of GJ proteins

Although a review on GJ protein evolution was published recently [68] and new updates in this field were discussed even more recently [69], the exponential growth of sequencing data from the genomes and complementary DNA (cDNA) of various organisms requires revision and refinement of our concepts. Comparison of genomes from different organisms suggested that similar functions are supported by related molecules derived from a common ancestor. In this respect, GJ proteins present an extremely interesting case for evolutionary and comparative analysis.

GJs are composed of membrane proteins that form a channel that is permeable to ions and small molecules, connecting the cytoplasm of adjacent cells (Fig. 1). They are considered to be a universal feature of all multicellular animals (*Metazoa*). Connexins were identified as the molecular components of vertebrate GJs and cloned more then 20 years ago [16,17]. Numerous attempts to clone connexins from invertebrates have failed, and finally, it was suggested that invertebrate GJs are assembled from proteins unrelated to connexins. This protein family was originally designated OPUS (acronym derived from the founding members ogre, Passover, unc-7 and shaking-B) [70]. It was suggested that these are specific invertebrate GJ proteins, and they were later renamed innexins (invertebrate analog of connexins [71]). After the presence of innexin homologues was established in vertebrates (PROSITE: PS51013), we proposed to reclassify innexins with their vertebrate homologs into a larger family, termed pannexins [19]. We suggested that pannexins are ubiquitous metazoan proteins, in contrast to the chordate-specific connexins. Analysis of recent sequence data from the genomes of different organisms largely supports these conclusions. However, important exceptions to this simplified scheme have emerged, and it appears that some animals have neither pannexins nor connexins at all, and some chordate species may have no connexins encoded in their genomes (Fig. 1e). At the same time, new genomic sequence data failed to reveal either connexin or pannexin homologs in prokaryotes, plants or fungi. This is consistent with the hypothesis that multicellularity in plants, fungi and animals emerged independently [72]. Although intercellular channels exist in all three living kingdoms, only animal channels are composed of proteins. Have GJs only emerged in multicellular organisms, or were these channels inherited from a unicellular ancestor, where they played a different role? The discovery of non-junctional (hemichannel) functions for both connexins and pannexins, which are essential for the physiology of a single cell, supports this possibility. Yet, no orthologs of these proteins are present in unicellular eukaryotes.

Choanoflagellata are the closest unicellular ancestors to multicellular animals. Thus, we have searched *Choanoflagellata monosiga brevicollis* whole-genome shotgun (WGS) raw sequence data of (over 650 million bp sequenced) for connexin- or pannexin-related sequences using the TFASTX algorithm, but failed to find either family. All bilaterian protostomes (e.g. flatworms, nematodes, arthropods, annelids and mollusks) likely harbor pannexins and lack connexins (Fig. 1c) [19,54,65,68-71,73,74]. The situation with basal metazoans is more complicated. Although pannexins were found in the ctenophore *Mnemiopsis leidyi* EST (expressed sequences tag) collection (NCBI gi:40547610) and in cnidarian *Hydra* [68,69,75], neither family was found in substantial sequence data on sponge *Reniera* [69] and in a limited database of placozoan *Trichoplax adhaerens*. Abundant genome sequence data on starlet sea anemone (*Nematostella vectensis, Anthozoa, Cnidaria*) also reveled no homology with either connexins or pannexins.

Thus, pannexins appear not to be truly ubiquitous in metazoans, although they are present in all bilaterian protostomes and chordates (Fig. 1e). Connexins have been documented in all vertebrates and also in

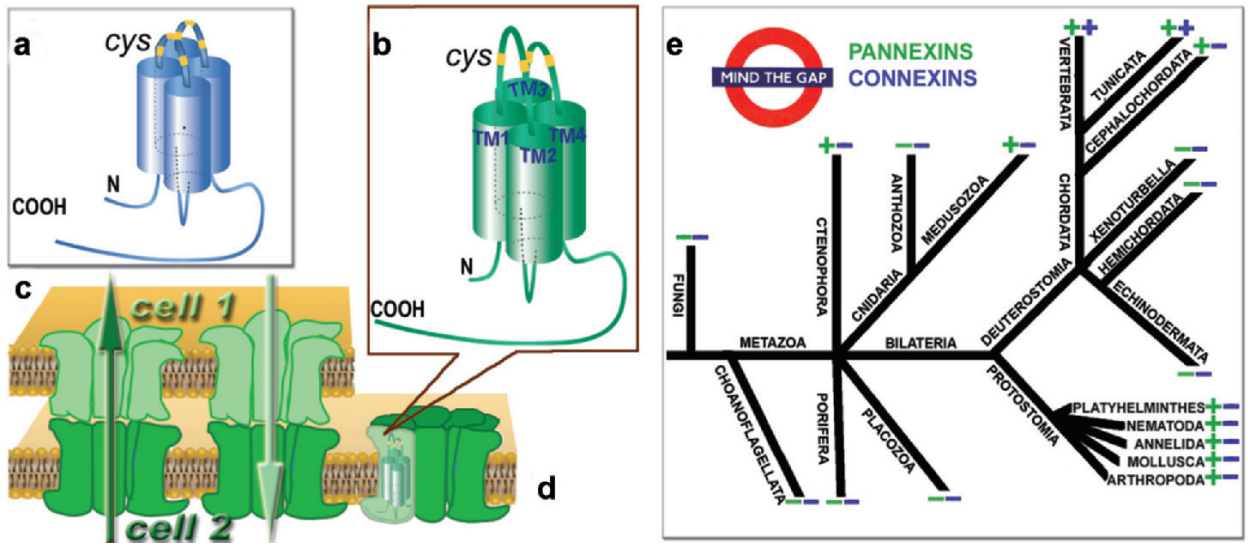

Figure 1. Artistic interpretation of 3D structure of typical connexin (*a*, blue) and putative topology of typical pannexin proteins (*b*, green), and the GJ channels (*c*) and the hemichannel (*d*) formed by these proteins in the membrane. Two regularly spaced cysteins (*cys*, yellow boxes) in pannexins and three cysteines in connexins direct interaction between extracellular loops and are required for docking of the opposing pannexons. The topology of connexins and pannexins with four transmembrane (TM) domains and intracellular N and C termini is rather similar, yet their sequences are not related. (*e*) Our current reflection on the presence (+) or absence (–) of connexins (blue) and pannexins/innexins (green) in the main taxonomic groups of Fungi/*Metazoa* division is indicated in a simplified phylogenetic tree. See text for details.

animals of the chordate branch, tunicates, ascidians and appendicularians [68,76,77]. Based on analysis of NCBI raw data, we suggested that both connexins and pannexins are absent in the sea urchin genome [69]. Recently published papers confirm our conclusion [78,79]. Hemichordates are also likely to be deprived of both connexins and pannexins, as about two-fold coverage WGS sequence data and substantial EST collection from *Saccoglossus kowalevski* does not contain homologs to known GJ proteins. Limited sequence data on *Xenoturbella* also contain no sequences related to connexins or pannexins. Three main groups are recognized within the chordates themselves: tunicates, cephalochordates or lancelets, and vertebrates. The search using TBLASTN or TFASTX algorithms for putative connexins in chordate animal lancelet in trace WGS data suggest that connexins are absent in its genome, although pannexins were reliably detected in the lancelet genome. This finding places vertebrates closer to tunicates, which have connexins, than to the lancelets (*Cephalochordata*) which are devoid of connexins. These phylogenetic relations between different chordate groups would have looked perplexing several years ago, yet recent studies also support them [80,81].

Remarkably, our comparative genomics studies suggest that some metazoans appear to have neither connexins nor pannexins (like the sea urchin, sea anemone, sponge and probably other animals indicated in Fig. 1e). This implies either that some multicellular animals have no GJs, or that an additional family(s) of GJ proteins does exist! Physiological data support the postulate regarding a ubiquitous presence of the GJs in *Metazoa*, including those species that lack identified GJ protein genes [82,83]. Subsequently, we predict the discovery of additional protein families recruited for GJ function in different organisms, potentially even in vertebrates.

The capacity of pannexins to form GJs: what do we know?

Known GJ proteins, both vertebrate and invertebrate, share a similar characteristic structure comprising four transmembrane domains spaced by two extracellular and one intracellular loop, and flanked by the intracellular amino- (NH2) and carboxy- (COOH) termini [84,85]. This structure is essential for the formation of a hexameric pore complex, the hemichannel (half-channel), which is the basic structural element of all GJs [22,84,86]. The extracellular loops of GJ proteins harbor several conserved cysteins, which are essential for intercellular docking of GJ hemichannels to counterparts in the apposing cell membrane [22,84]. The resultant membrane-spanning molecular channel bridges the narrow gap between neighboring cells and interconnects two cytoplasms. As with other GJ proteins, all three members of the vertebrate pannexin family, PANX1, PANX2 and

PANX3, also possess four transmembrane domains and two conserved cysteins in the extracellular loops [18] (Fig. 1b). These structural similarities encouraged investigators to assign the GJ role to pannexins [18,19,56]. More extensive homology between vertebrate pannexins and invertebrate pannexins/innexins extends further to extracellular loops possessing two conserved cysteine residues, which is different from connexins harboring three cysteins per loop [18,19,87]. In contrast to connexins possessing relatively small (~30 aa) extracellular loops (Fig. 1a), many innexins and pannexins share an increased (50–68 aa) size of their extracellular loops (Fig. 1b), which may apparently be reflected in the strength of interaction between opposing hemichannels. However, given the shortage of relevant ultrastructural and biophysical data, it is premature to speculate on this issue. Does the molecular structure of pannexins facilitate the formation of a canonical hexameric membrane complex and a functional membrane-spanning pore? Classical functional assays for GJ proteins include formation of low-resistance electrical coupling and capability to mediate transfer of small (< 1000 Da) tracer molecules between cells expressing GJs [28,31]. Bruzzone and co-authors showed that in paired *Xenopus* oocytes rat Panx1 when expressed alone or in combination with Panx2 induces the formation of intercellular channels [56]. More evidence of GJ formation by pannexin molecules derives from independent experiments, where rat Panx1 properties were studied in C6 glioma cells and properties of human PANX1 in prostate cancer epithelial LNCaP cells. Loading Panx1-expressing cells via dialysis from a patch pipette with a fluorescent SR101 dye showed significant coupling with neighboring cells, evidence of functional intercellular channels [27]. Coupling between C6 cells stably expressing Panx1, Panx1-GFP or Panx1-myc has further supported the ability of Panx1 to form functional GJs. In LNCaP cells, expression of fusion PANX1-EGFP protein was detected in the endoplasmic reticulum (ER) and plasma membrane and resulted in formation of channels permeable to Ca^{2+} ions. In the junctional area of cell-cell contacts, GFP (green fluorescent protein) fluorescence localized to a fine punctate staining, interpreted by the authors as GJ-like aggregates [62]. The characteristic labeling pattern was associated with the newly acquired permeability to Ca^{2+}, and suggested that Panx1-GFP formed patent GJs coupling to neighboring LNCaP cells. In contrast, somewhat similar Panx1-specific antibody labeling detected in transfected HeLa cells has been interpreted differently: very fine puncta evident in junctional plasma membrane was considered as discrete from typical GJ plaque-like staining [64]. Despite contrasting interpretations of the immunohistochemistry data, functional studies revealed convincing evidence that pannexins are able to form patent GJs between cells *in vitro*. Distinct properties and labeling patterns of pannexins in junctional membranes may suggest different morphology of GJ plaques formed by these proteins. Interestingly, small rosette-like GJ plaques were previously documented by ultrastructural analysis in crayfish axons (that express pannexins/innexins) by Peracchia [88,89]. Smaller plaques are also identified in rodent spinal cord [90]. We speculate that immunolabeling of this morphologicaly distinct, small rosette-like type of GJ plaque should differ significantly from the bright punctuate staining typical of connexin-type GJs. If this hypothesis is true, the fine puncta that were observed in the cell junctional area of HeLa and LNCaP cells may well represent the labeling specific to GJs formed by pannexins. To date, pannexins have not been demonstrated in ultrastructurally defined GJs or any other membrane structure in vertebrate species. In addition, impedance studies of connexin knockout strains produced convincing evidence that almost 100% of the electrical coupling in the ocular lens could be attributed to connexin GJs [91,92], thus suggesting minimal if any contribution for the abundantly expressed pannexins [93]. Consequently, major uncertainties continue to preclude unambiguous interpretation of physiological, biochemical and immunohistochemical studies of the role and subcellular distribution of pannexins. Despite existing *in vitro* data that attribute GJ function to pannexins, such GJs remain yet to be shown *in vivo*.

Until recently, all GJ functions in vertebrates were considered to be contributed by connexins. With the discovery of pannexins that possess significant overlap in expression profile with connexins, this paradigm needs to be revisited. Both families of GJ molecules are co-expressed in many types of vertebrate cells, a fact that complicates discriminating the contribution of each individual protein species to either GJ or hemichannel function. High sensitivity of both pannexins and connexins to carbenoxolone (CBX) and flufenamic acid (FFA) [56], drugs routinely used to block hemichannels, and lack of specific blockers further complicates the task of discriminating between the two channel families. Indeed, the activity of previously unknown but ubiquitously expressed pannexin channels may have affected earlier experimental results that were attributed entirely to connexins. Such data might now require critical re-evaluation [58]. Given that many connexin knockout mouse strains have been successfully generated, such re-evaluation might be be considered once pannexin knockouts become available.

Non-junctional functions: pannexin hemichannels

Recent studies have presented evidence of the activity of Panx1 hemichannels, whereas experimental proof of GJ function of the endogenous protein is still missing. Hemichannels are defined as large, non-selective ion channels that reside in the non-junctional plasma membrane before their assembly into GJ channels [94]. The magnitude of hemichannel contribution to the passage of ions and metabolites across the membrane depend on their intrinsic properties, gating and ability to gate into the open configuration, and on the dwell time in the non-junctional membrane. The lack of change in the conductance current after cell contact in the paired oocytes expressing pannexins presents evidence for slow dynamics of pannexin–mediated coupling, extended dwell periods for pannexons and no tendency to increasing percentage of docked vs. uncoupled hemichannels [57]. This is in contrast to the typical behavior of connexins, which show a profound propensity to progressively incorporate into GJ channels once two cells are paired together [48,95]. The lack of docking 'vigor' implies that high proportions of pannexin hemichannels coexist with full channels in the same cells, and emphasizes the biological role for non-junctional pannexins [57,61]. Accordingly, studies of the tumor-suppressive phenotypes acquired by Panx1-transfected C6 glioma cells revealed both GJ-dependent along with GJ-independent effects of pannexin channels [27]. A distinct hemichannel activity has been observed by many researchers in cells expressing pannexin1 [57,59,61,96,97].

Channel properties

Historically, functional specialization of seemingly redundant connexins was confirmed by the coexistence of multiple family members with distinct expression profiles, non-redundant contribution to growth and development, diverse nature of diseases caused by their mutations and high selectivity of the homo- and heterotypic channels they form [12,29]. The physiological properties of vertebrate pannexin channels are distinct from those of connexins and ensure that unique, rather than a redundant cellular role can be assigned for this protein family. Upon activation, pannexons open into large non-selective pores which are insensitive to physiological levels of extracellular Ca^{2+} but permeable to ions and small molecules and metabolites up to 1000 Da in a wide range of membrane depolarization levels [57,60]. The ability of pannexins to form patent membrane-spanning hemichannels and the gating properties of these hemichannels have been studied in *Xenopus* oocytes [56,57], and human [62] and rodent cell lines [64]. Substantial voltage-activated currents were consistently induced when single oocytes expressing Panx1 were stimulated by voltage steps >20 mV, while neither Panx2 nor Panx3 induced membrane currents under the same conditions when expressed alone. Pannexin hemichannels differ from typical connexin channels by exhibiting larger currents with increasing depolarization, faster kinetics of pore opening, larger unitary conductance (>500 pS, compared to a maximum ~ 300 pS with connexins), very weak voltage gating and multiple substates in single-channel recordings [56,60]. Similar to heteromeric connexin hemichannels, heteromeric Panx1/Panx2 hemichannels exhibited distinct properties: reduced current amplitude and modified voltage-gating kinetics with respect to homomeric Panx1 channels [56]. The ability of the Panx1 protein to form both homomeric channels and to interact with Panx2 to form functional heteromeric channels has been demonstrated in paired oocytes [57]. Currents recorded from oocytes coexpressing Panx1 with Panx2 were smaller and significantly delayed in achieving a peak current compared to currents measured in cells injected only with Panx1 mRNA, indicating that the presence of Panx2 modified properties of heteromeric hemichannels. Analysis of amino acid sequences of human and rodent pannexins revealed a single predicted phosphorylation site for pannexin1 and multiple putative phosphorylation sites for pannexin2. By similarity with the Cx43 channel, which is known to change permeability in response to phosphorylation status [98-100], we can hypothesize that phosphorylation is likely to affect the properties of heteromeric Panx1/Panx2, rather than homomeric Panx1/Panx1 channel [87].

Despite general parallels with connexins, the pharmacological properties of pannexin hemichannels are distinct. As compared to connexin 46 (Cx46) hemichannels, both homomeric and heteromeric (Panx1/Panx2) channels show significantly higher sensitivity to CBX but remain relatively insensitive to FFA, the two commonly used GJ blockers [57]. In addition, CBX affects both current amplitude and kinetics of channel closure, whereas FFA only inhibits peak current values. A spectrum of stimuli that regulate pannexon opening (Fig. 2) is also distinctive [24-26,59-61,87], providing a feasible explanation for coexpression with connexins observed in many vertebrate cell types [18,56,93,101-104]. To date, mechanical stress, positive membrane potential, extracellular ATP, elevation in intracellular Ca^{2+} ($[Ca^{2+}]i$), ischemic insult and inflammation have been shown to activate pannexon opening, while cytoplasmic acidification with CO_2 was efficient in closing this channel [59]. Although mechanosensitivity has been demonstrated

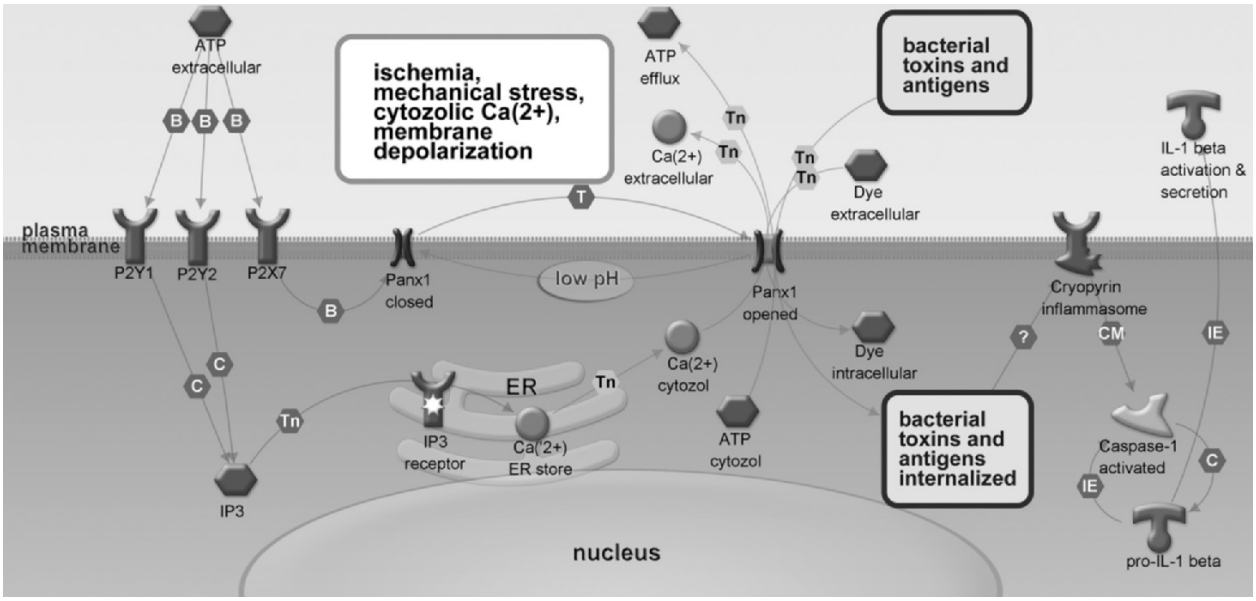

Figure 2. The pannexin1 hemichannel is activated (T, transition into open configuration) via stimulation of metabotropic (P2Y) and ionotropic (P2X) purinergic receptors, increase in cytosolic Ca^{2+}, membrane potential, mechanical stimulation and some pathological stressors. Opened channels are capable of passing dye, Ca^{2+}, ATP and other small molecules, including bacterial toxins and antigens. Low intracellular pH and conventional hemichannel blockers close the channel. Green arrows, activation; grey arrows, translocation; B, binding; Tn, transport; C, cleavage; ?, unspecified mechanism; CM, covalent modification; IE, influence on expression.

for Cx46 [60], and Cx43, Cx37, Cx35, Cx32, Cx26 have been widely implicated in ATP-induced ATP release [21,94,105-110], very few connexin hemichannels (except for Cx32 and Cx30.2 [53,111]) remain open on elevation of [Ca^{2+}]i, exposure to the physiological (millimolar) range of extracellular calcium ([Ca^{2+}]o) and membrane depolarization, as do pannexons [15,59,112,113]. The majority of connexons pass Ca^{2+} and ATP at very low [Ca^{2+}]o [108], but become closed at 1–2 mM levels [113]. In addition, the demonstrated ability of the Cx43 channels to pass ATP and propagate Ca^{2+} waves in non-contacting cells has been compromised by experiments with Cx43 null astrocytes, which sustain the normal speed of Ca^{2+} wave propagation [114]. In contrast, pannexin1 hemichannels opened by an increase in intracellular Ca^{2+} and supported rapid ATP release, capable of generating the ATP-specific current across the membrane [59,60]. These differences in properties between pannexons and connexons may indicate that pannexin hemichannels are suited for ATP transport across the membrane in a wider range of physiological conditions, which is critical for supporting efficient ATP release in non-contact Ca^{2+} wave propagation.

The capacity of pannexin hemichannels to open through an increase in intracellular Ca^{2+} may also apply to putative properties of GJ channels made of pannexins. Indeed, experiments performed by Vanden Abeele and co-authors in the human prostate cancer LNCaP and HEK-293 cell lines [62] demonstrated that expression of Panx1-GFP fusion protein enabled formation of GJ channels between adjacent cells, which mediated direct intercellular diffusion of Ca^{2+}. In addition to facilitating intercellular transport of ions and molecules, overexpression of human *PANX1* increases Ca^{2+} permeability of the ER (endoplasmic reticulum) membrane, whereas PANX1 depletion by antisense and small interfering RNA (siRNA) strategy inhibited such permeability, causing an increase in the Ca^{2+} content of the ER [62]. Combined with abundant expression of Panx1 in the ER in multiple cell types [62,64,93,97,103,115], these data provide new insights into molecular mechanisms of the basal ER Ca^{2+} leak, which have been poorly understood.

With the lack of *in vivo* evidence of GJs formed by pannexins, characteristics of pannexin channels have encouraged some investigators to question their gap junctional role and to suggest that these proteins were preserved throughout vertebrate evolution to perform exclusively non-junctional functions [51]. Although critical ultrastructural data on membrane-bound pannexins is still missing, new data that support the ability of human pannexins to form GJs has become available [62]. While pannexin hemichannel function has been demonstrated *in vivo*, we speculate that pannexin GJs can also play an important role in vertebrate tissues.

The role of pannexin1 in Ca^{2+} wave propagation

Both localization data (see below) and functional characteristics of hemichannels formed by pannexin1 suggest that they are perfectly tuned to promote the propagation of calcium waves. The role of GJs in Ca^{2+} wave propagation was considered long before the discovery of pannexins. Connexins, the only known GJ molecules at the time, are highly sensitive to physiological (500–600 nanomolar, up to a micromolar) increase in [Ca^{2+}]i [112,116,117]. It was therefore proposed that connexins contribute to Ca^{2+} wave propagation by intercellular transfer of inositol trisphosphate (IP3) at the early stage of wave generation, prior to connexon closure by rising intracellular Ca^{2+} [118-120]. These properties of connexin GJs could limit their capacity to participate in Ca^{2+} wave propagation, and emphasize the potential role of pannexin channels, which appear to be better "tuned" for this function [57,59,62].

Experimental evidence that pannexons work in synergy with metabotropic purinergic receptors to support the ATP-induced ATP release came from studies on *Xenopus* oocytes. The ability to release ATP upon stimulation with extracellular ATP is the central event of regenerative Ca^{2+} wave propagation in many cell types [121]. Locovei and co-authors reconstructed this combination and examined ATP release in oocytes coexpressing PANX1 and either P2Y1 or P2Y2 receptors [59]. While no ATP current was detected in oocytes expressing PANX1 alone, application of ATP to oocytes coexpressing either PANX1/ P2Y1 or PANX1/ P2Y2 pairs resulted in large transmembrane ATP currents [59]. The kinetics of ATP release differed depending on the receptor used: P2Y1 activated ATP release slowly, and the response was sustained over a period of ATP application. In contrast, P2Y2 generated a fast and transient response, consistent with the fast kinetics of Ca^{2+} wave propagation facilitated by this receptor [122]. These findings convincingly establish that P2Y purinergic receptors functionally coupled to the Panx1 channel represent sufficient molecular machinery for ATP-induced ATP release [59]. Ionotropic (ligand-gated) purinergic receptors, including $P2X_7$, were also shown to be suitable for this role [97]. The molecular mechanism of the pannexin hemichannel opening remains obscure. Binding to $P2X_7$ purinergic receptors revealed by immunoprecipitation analysis in HeLa cells [25] suggests that Panx1 hemichannel opening in response to extracellular ATP can be triggered via direct stimulation by activated receptors. Alternatively, pannexin hemichannel opening can occur indirectly, as a result of intracellular Ca^{2+} release caused by activation of metabotropic P2Y purinergic receptors that stimulate production of IP3 [59].

Evidence that the Panx1 hemichannel opening is regulated by stimulation of various purinergic receptors has been obtained in studies of signal transduction in taste buds and macrophages [24,25,96,97] and will be discussed below in more detail. Pannexin-mediated ATP release is likely to be implicated in Ca^{2+} wave propagation in blood endothelium, causing NO-mediated smooth muscle relaxation and subsequent dilation of blood vessels [123,124]. Such dilation typically occurs in response to mechanical (shear) stress or ischemic conditions in the tissue. Blood endothelial cells are probably capable of ATP release in response to mechanical stress both via Cx43 and Panx1 hemichannels [61]. Accordingly, the presence of Panx1 in blood endothelial cells has been demonstrated by immunohistochemistry [61,93] (and Fig. 3, insert). An additional mechanism implicating pannexin channels in vascular perfusion regulation has been suggested. It relies on a well-known ability of human erythrocytes to release ATP in response to ischemia [125,126] and mechanical stress [127], which subsequently trigger Ca^{2+} waves in the endothelium [61]. The fact that erythrocytes do not form vesicles, express connexins or form GJs allowed Locovei and co-authors [61] to single out pannexin hemichannels as conduits for ATP release from erythrocytes in this mechanism. The above-mentioned properties of pannexons allowed us to attempt a reconstruction of their putative roles in intercellular Ca^{2+} signaling and wave propagation in blood capillaries (Fig. 3). Mechanical (shear) stress and ischemia open pannexin hemichannels both in endothelial cells and erythrocyte membranes, enabling the exit of cytoplasmic ATP. Extracellular ATP binds to metabotropic P2Y purinergic receptors on the surface of blood endothelial cells, which triggers an increase in IP3 production and a release of intracellular Ca^{2+} [119]. Open pannexon channels also allow for extracellular Ca^{2+} influx. The compound effect of this influx and IP3-mediated Ca^{2+} release causes an increase in intracellular Ca^{2+}, which may contribute to Ca^{2+} wave propagation either via intercellular IP3 signaling using connexin GJs (classical GJ-mediated pathway) or via pannexin GJs (pannexins GJ-mediated pathway), if their existence is confirmed in vivo. At the same time, extracellular diffusion of the ATP released by pannexin channels promotes Ca^{2+} wave propagation by a GJ-independent mechanism [59,61]. Given that neighboring cells synchronously trigger $Ca^{2+}$and IP3 release and ATP secretion, the robust propagation of a Ca^{2+} wave capable of traveling a long distance is ensured with and without direct cell contact [21,94,106].

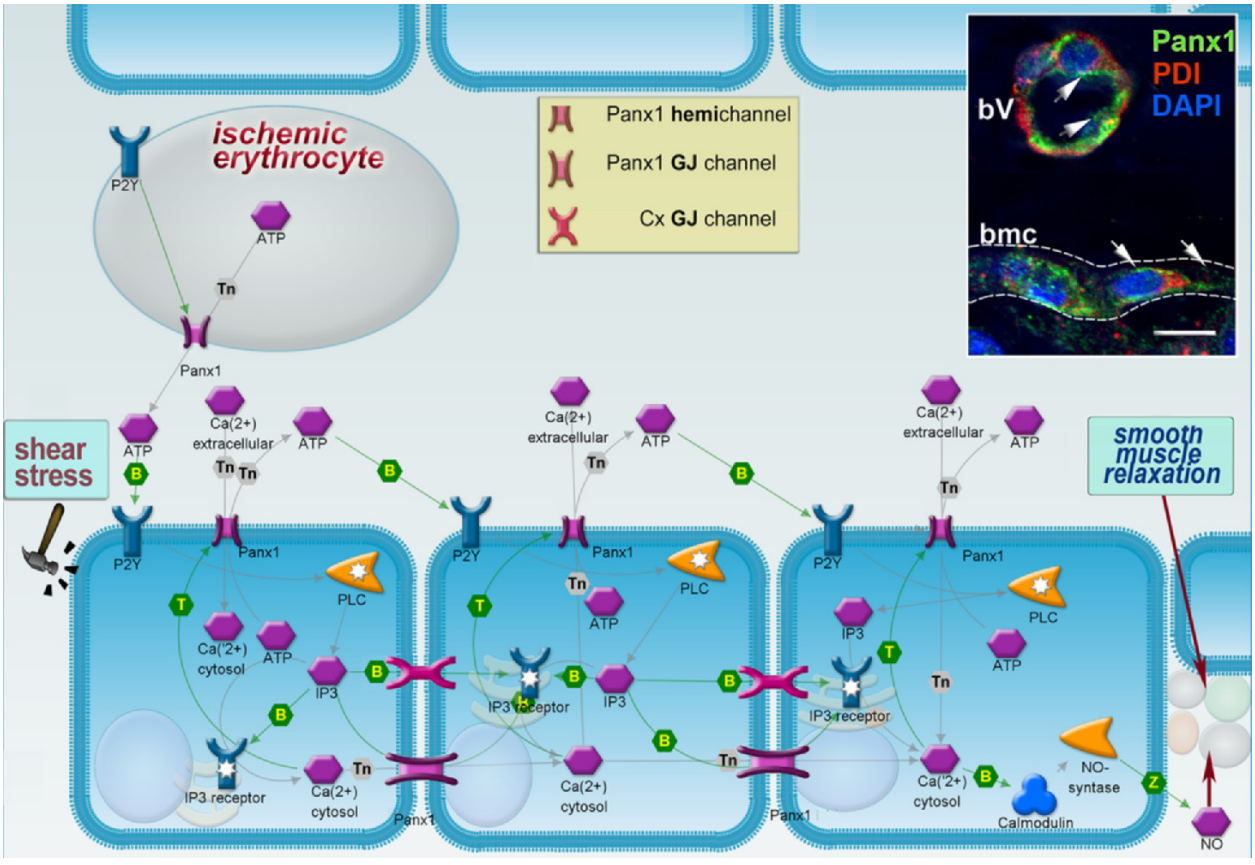

Figure 3. Scheme depicting putative mechanisms of Ca^{2+} wave propagation in blood endothelium that trigger NO (nitric oxide)-mediated dilation of the blood vessel. Pannexons are permeable to IP3, ATP, Ca^{2+} ions and are capable of supporting the two putative mechanisms of Ca^{2+} wave propagation in blood endothelium: 1) an extracellular mechanism that requires ATP release into the blood, and 2) intercellular, via direct IP3 and Ca^{2+} ion transport through pannexin GJ channels. In response to low oxygen, and/or mechanical stimulation, pannexin1 hemichannels in the plasma membrane of erythrocytes open and release ATP, which binds to the purinergic P2Y receptors at the surface of endothelial cell (blue boxes) membrane. This triggers IP3-mediated release of Ca^{2+} from internal calcium storages, which, in turn, facilitates opening of pannexin hemichannels and release of ATP into the external medium and directs Ca^{2+} diffusion to adjacent endothelial cells via pannexin GJ channels. Secreted ATP affects the neighboring cells and promotes Ca^{2+} wave by a paracrine mechanism. Alternatively, pannexin1 hemichannels can open in endothelial cells in response to shear stress, thus allowing inflow of extracellular Ca^{2+} ions into the cells. See text for details. B, binding; T, transition into open configuration 1; Tn, transport; Z, catalysis; ?, unspecified mechanism of activation; white star is for IP3 receptors activated for Ca^{2+} release from the ER. Arrows: green, activation; grey, transport and catalytic reactions. Scheme adopted with modifications from [61], see text for details. (***Insert***) Typical labeling of inner retina blood vessels (**bV**) and microcapillaries (**bmc**, dotted lines) using polyclonal anti-human PANX1 antibodies (c-terminal) in mouse retina slices [103]. Specific labeling for PANX1 (green) localized to the luminal side of blood endothelial cell membrane (arrows) and to the ER. Partial co-localization with the ER, stained with anti-protein disulfide isomerase (PDI, in red) antibodies, was observed in perinuclear region. The image was obtained in this study. Nuclei are labeled with DAPI (blue); bar, 10 μm.

Pannexin expression

Initial analysis of the human genome for homology with the innexin family of invertebrate GJ proteins revealed open reading frames (ORFs) for three genes, *PANX1*, *PANX2* and *PANX3*, with calculated molecular masses of 48, 73 and 45 kDa, respectively [18,19]. The *PANX1* gene was located on human chromosome 11q21, *PANX2* on human chromosome 22q13 and *PANX3* on chromosome 11q24 [18]. The protein-coding regions were assigned to five, three and four exons, for these three genes. *PANX1* has two splice variants that differ in the fifth exon; *PANX2* has two alternatively spliced (messenger RNA) isoforms; and isoforms for *PANX3* were not detected [18,64]. Transcripts for *PANX1*, the most ubiquitously expressed gene, were detected in numerous tissues, including brain, spinal cord, skeletal and heart muscles, eye, thyroid, kidney, prostate, blood endothelium, erythrocytes and others [19,56,101,102,104,128]. *PANX2* showed more restricted expression in human, where it was predominantly brain-specific [18]. In rodents, the *Panx2* mRNA co-expressed with *Panx1* in many organs, including brain, eyes, spinal cord, prostate, pancreas, liver and kidney [69,87,93] (see also GEO# GDS426 at http://www.ncbi.nlm.nih.gov/geo/). Ac-

cording to the initial characterization, *Panx3* expression was limited to skin and cartilage in the mouse [18,56,69]. In the rodent central nervous system (CNS), transcripts for *Panx1* and *Panx2* are widely detected in retina, cortex, hippocampus, cerebellum, inferior olive, olfactory bulb and spinal cord, with *Panx1* mRNA expressed primarily in neurons, less in Bergman glial cells and endothelial cells, and possibly in oligodendrocytes/CNS myelin [18,56,101-104,129]. High levels of expression of *Panx1* in adult skeletal muscle and heart was rather unexpected for a GJ protein. To some extent, this can be explained by the contribution from blood endothelial cells, erythrocytes and macrophage abundantly present in this tissue [24,25,61,93]. Further research is required to determine localization and function of pannexins in these tissues.

Pannexin1 and, especially, Pannexin2, which is, presumably, brain-specific in humans, received significant attention from investigators in the field of electrical synapses and were scrutinized for their role in intercellular communication in brain neuronal circuits [101,102,129]. Pannexins were readily suggested to fill several gaps in the CNS network electric coupling "puzzle". Thus, during early development GJs of unknown composition and calcium signaling were shown to be essential for synchronizing cell proliferation and migration [130,131]. Abundant pannexin expression in embryonic brain and pannexin channel properties make these proteins apparent candidates. Developmental patterns of the expression of both family members have been studied in rodent brain and eyes by *in situ* hybridization and immunohistochemistry (IHC). These studies showed widespread and mostly parallel accumulation of both mRNAs, but revealed that *Panx1* and *Panx2* are inversely regulated during the development of the rat brain. In the mouse, *Panx1* showed high levels of expression in the embryonic and young postnatal brain and declined considerably in the adult, whereas *Panx2* mRNA was low in the prenatal brain but increased substantially during subsequent postnatal development [101,102,104]. These results have been confirmed by IHC data showing the most prominent accumulation of Panx1 in neonatal retinal neurons [103]. Similar patterns of expression have been detected in the ocular lens. This tissue possessed peak accumulation of the *Panx1* and *Panx2* transcripts in undifferentiated lens epithelial cells in the mouse [93]. Both RT-PCR and *in situ* hybridization data indicated that the transcript abundance declines as lens epithelial cells progress with differentiation into mature fibers. The observed evidence suggests the involvement of homomeric (Panx1/Panx1) channels in early development, while the function of heteromeric (Panx1/Panx2) channels, characterized by lower macroscopic currents, might be important in fully differentiated adult tissues [57,87].

Expression of alternative GJ protein in the CNS received much attention from researchers interested in electrical circuitry in neuronal networks synchronization of the network activity, and was extensively reviewed elsewhere [69,87]. High-resolution mapping uncovered abundant expression in the adult cortex, hippocampal and neocortical pyramidal cells, and interneurons, neurons of the reticular thalamus, the inferior olive, magnocellular hypothalamic neurons, midbrain and brain stem motoneurons, Purkinje and Golgi cells in the cerebellum [56,101,104,115], and horizontal and ganglion cells in the retina [56,103]. Only a few brain cell types, like the noradrenergic cells of the locus coeruleus, raphe magnus and raphe pallidus lacked pannexins [115]. Several intriguing observation were made in these studies. First, pannexin expression was found in brain regions devoid of connexins but known to be electrically coupled, like hippocampal pyramidal cells, indicating a possible contribution to the pool of electrical synapses [128]. Second, expression of *Panx1* in both electrically coupled and uncoupled cells [115] suggests that a function other than that of GJs should be considered for this protein. In this regard, it is noteworthy that apart from Purkinje and pyramidal cells that showed punctuate cell surface labeling, other CNS neurons exhibited predominantly cytoplasmic labeling [64,103,115]. Third, despite a significant overlap, the *Panx1* expression pattern was distinct from that of *Panx2*.

The functional significance of the expression data relates to the role that GJs and hemichannels play in electric coupling, synchronization of activity and second messenger signaling in the brain. Spontaneous and sensory-driven activity is important for both cortical development (neuronal differentiation, migration, synaptogenesis, neurotransmitter specification etc.) to shape functional neuronal circuits, and in adults, where it is organized in distinct spatiotemporal patterns and is essential to sensory binding, synaptic plasticity, memory processing and large-scale integration [132-135]. The abundance of pannexins in the Purkinje cells, hippocampal pyramidal cells and Bergman glia served to suggest that they may contribute to electric coupling and generation of ultra-high-frequency (150–200 Hz) oscillations in the hippocampus and cerebellum [104,115]. These cells are coupled by electrical synapses but do not express Cx36 and Cx45 [136], suggesting pannexin-mediated coupling as a plausible alternative to connexin GJs. Significantly, the ultra-high-frequency oscillations that are dependent on electrical coupling between cells remain intact

in these regions in the mouse strain with a genetically impaired Cx36 gene [137,138]. Dysregulation of the ultra-high-frequency oscillations is observed in epilepsy [133,139], a disease with poorly defined molecular determinants, characterized by seizures and loss of brain neurons. Initial studies of pannexin1 expression in rat brain following injections of 4-aminopyridine, which results in systemic seizures mimicking epilepsy, failed to reveal the loss of *Panx1*-expressing neurons. In contrast, a decline in *Cx36* expression was clearly observed in these animals, allowing the authors to suggest that these two GJ proteins are involved in different functional roles or that they are expressed in different cell types in the brain [115].

The pannexin expression data in glial cells, known to utilize both GJ and hemichannel activity to coordinate metabolic responses in their networks, remain controversial. Gene expression studies were not consistent in detection of the *Panx1* message in the white matter [102,128], once reported by Bruzzone and co-authors [56]. Later examination documented *Panx1* expression only in Bergmann glia and Golgi epithelial cells, but did not confirm the earlier observations in the white matter [115]. Combined with a lack of specific labeling in the white matter and co-localization with cells expressing astrocyte markers (glial fibrillar acidic protein, GFAP), these results were suggestive of either low level or rather selective expression in certain glial types [56,101,102,115,128]. In contrast, recent studies that have revealed endogenous expression of all three pannexin genes in rat primary astrocytes [27]. The IHC analysis of *Panx1* expression in primary astrocytes, oligodendrocytes and hippocampal neurons showed both the transcript and the protein accumulation in mouse cells. Glial cells are known to express at least six different connexins that represent a conduit to sustain extensive glial networks [140,141] and, potentially, limited glia-neuron coupling [142]. Huang and co-authors reported that the Panx1 immunoreactivity did not co-locolized with GJ plaques in cultured oligodendrocytes or astrocytes, and suggested strictly non-junctional role for glial pannexin1 [64].

Intracellular localization

Panexin1 protein, the only member of the family capable of forming homomeric channels [57], is detected in both plasma membranes and, much more abundantly, in the cytoplasm [27,62,115]. In particular, experiments using transient expression of this protein in cultured cells [27,62,64,93,103] showed abundant accumulation in the Golgi apparatus and the ER. One feasible explanation for the ER and Golgi labeling implies that posttranslational modification and assembly of pannexons share the same route demonstrated for connexins [143,144]. The ER-bound Panx1 was, therefore, suggested to be either a pool of unprocessed precursor proteins [64,103] or assembled functional pannexons that serve as ER calcium release channels, thus providing a conduit for the enigmatic Ca^{2+} leak across the ER membrane [62]. A similar pattern of organelle labeling was observed in primary cultures of rodent astrocytes, oligodendrocytes and hippocampal neurons probed by peptide anti-Panx1 antibodies [64,103]. All cultured glial cells, except immortalized human cells transfected with Panx1 expression construct [27,62,64,115], were negative for membrane staining [64,93,103]. IHC studies in rodent brain slices revealed only Purkinje and hippocampal pyramidal neurons showing cell surface accumulation of Panx1 protein, whereas the majority of neurons and glial cells showed profound cytoplasmic staining pattern [64,103,115].

Membrane staining was prominent in rodent blood endothelial cells (Fig. 3, insert), mature lens fiber cells, but not in young fibers and undifferentiated lens epithelial cell [93], retinal or brain neurons [103,115], where it localized to dendrites. A disproportionate accumulation of Panx1 in organelles rather than in plasmalemma has been detected with several anti-Panx1 antibodies in neurons and other cell types, and is rather unusual for a GJ protein. As with many membrane integral proteins, potential for the existence of multiple Panx1 isoforms, epitope masking issues and narrow specificity of peptide antibodies is likely to complicate the task of defining intracellular localization. With more antibodies generated in different laboratories, this is likely to be resolved in the future. Alternatively, an active role in the ER provides an indication of intracellular, non-junctional function for pannexons.

At the time when this manuscript was being submitted, the first TEM (transmission electron microscopy) data detailing intracellular localization of Panx1 became available. Zoidl and co-authors presented evidence of pannexin protein accumulation in the plasma membrane and ER of post-synaptic neurons in the CA1 region of mouse hippocampus [145]. Asymmetric localization of immunogold labeling in the synapses was consistent with hemichannel, rather then GJ function of pannexin1.

What systemic roles do pannexins play?

As chordates apparently possess two distinct types of GJ molecules, it is important to understand what is the balance of function between them. Do pannexins

duplicate GJ functions of connexins, or do each of them play distinct physiological roles? Unique properties of pannexin channels, which can be opened by intracellular Ca2+ increase, pro-inflammatory stimuli or stimulation of purinergic receptors, encouraged assignment of putative roles in the propagation of glial and blood endothelial Ca^{2+} waves, vasodilation [59], ischemic cell death, inflammatory response [24-26,61] and release of synaptic neurotransmitters in taste buds [96,97]. Here we will review experimental data relevant to these unique, non-redundant functions of pannexin channels.

Pannexin channels facilitate paracrine signaling

The putative role that has been assigned to pannexin channels in paracrine signaling in vascular endothelial cells has been described above. Paracrine secretion of glutamate and ATP is believed also to be the basis of long-range Ca^{2+} signaling between astrocytes, and astrocytes and neurons in the CNS [146,147]. As compared to synaptic transmission, paracrine signaling is rather robust: it is independent of extracellular Ca^{2+}, vesicular transport and close cell opposition [106]. It also requires minimal molecular machinery, which was demonstrated by Bao and co-authors, who reconstructed functional ATP-gated ATP release in frog oocytes [59]. These experiments demonstrated that co-expression of genes encoding P2Y receptor and Panx1 in the same cell is a necessary and sufficient requirement for non-selective hemichannel pore opening in response to ATP stimulation. Hemichannels allow passage of ions and small metabolites (up to 1 kDa), including ATP, across the membrane [21,46,51,94]. This property has been implicated in the long-range intercellular signaling in a form of Ca^{2+} waves originally described in C6 glioma cells [105,108]. In addition to the 'classical' scheme of Ca^{2+} wave propagation, which implies direct cell contact and functional GJs [148], the release of ATP into the extracellular space provides an alternative, non-contact route for such signaling, as we described earlier. Studies of hemichannels, however, are fraught with opportunities for misinterpretation as being sometimes solely based on pharmacological inhibition with non-specific channel blockers, physiological measurements and on the erroneous assumption that connexons are the sole contributors to this activity [58]. Despite evidence that connexin hemichannels were demonstrated to facilitate ATP-dependent ATP release ([21,108,149,150]), channel properties of pannexons, particularly activation by intracellular Ca^{2+}, insensitivity to physiological concentrations of extracellular Ca^{2+}, mechanosensitivity, permeability to ATP and ability to open following stimulation of purinergic receptors, argue that these channels are physiologically better suited to function as ATP pores when compared to connexons [57,59-61,87]. Gated ATP release via the Panx1 hemichannels has also been shown to occur upon activation of ionotropic P2X receptors in response to gustatory stimulation in mammalian taste buds [96,97] and during early stages of inflammatory response [24,25].

Other possible functions in CNS and PNS

Although pannexin expression in glial cells is not as robust in comparison with neurons, their role as ATP-permeable pores could be important for long-range Ca^{2+} signaling in glial networks. Recent studies in rat primary astrocytes revealed endogenous expression of all three pannexin genes, which was not the case in C6 glioma cells [27]. Significantly, transfection of these tumorogenic cells with exogenous *PANX1* suppressed cell proliferation and reversed morphology from spindle-shaped to flattened, suggesting a role of pannexin1 in suppressing oncogenic transformation.

Among other important CNS functions, pannexons were suggested to represent a conduit for a negative feedback loop between horizontal cells (HCs) and cones in the retina [103] that was previously assigned to the connexin hemichannels [151]. HCs convert excitatory chemical information (presynaptic gluatamate release) from the cones in the center of receptive fields into an inhibitory signal for surrounding cones, thus enlarging receptive fields and contributing to signal contrast. These neurons showed expression of *Cx26* in the carp retina [151], and *Cx57* and *Panx1* in the mouse retina [103,152]. Although functional studies including tracer coupling and receptor field measurement confirmed that Cx57 mediates GJ coupling [153], increased accumulation of Panx1 in developing HCs and a punctate labeling pattern in the vicinity of adult HCs [103] are consistent with putative role for Panx1 hemichannels in the negative feedback pathway.

Taste cell signaling

Gustatory receptor cells within taste buds detect sweet, bitter and umami tastants via G-protein-coupled taste receptors. Because ATP has been recognized as a key neurotransmitter between Type I sensory and Type II output cells in the taste signal transduction cascade [154], both classical vesicular secretory system and hemichannel pathways were scrutinized for their role in taste sensory transduction [155].

Although detailed transduction mechanisms downstream of such receptor cells have been elucidated before, understanding the signaling from these cells to

the afferent nerve remains limited. Physiological recordings performed in individual taste bud cells suggest a Ca^{2+}-independent and voltage-dependent mechanism of ATP release in response to stimulation with sapid molecules, which implies activity of either pannexin or connexin hemichannels [96]. A more recent study by Huang and co-authors [97] excluded the possibility of a connexin-mediated secretory pathway and presented convincing experimental evidence that pannexin 1 hemichannels form a functional ATP sensory/release unit in Type II (receptor) cells to transmit signals to multiple Type III cells present in taste buds. These studies document that Panx1 hemichannels underlie ATP secretion from taste receptor cells and explains the lack of conventional synaptic features in these cells. Neurotransmitter secretion from taste receptor cells via Panx1 also provides the missing link between chemosensory transduction and expression of TRPM5 cation channels in receptor cells. Intracellular calcium release evoked by taste stimulation opens TRPM5 channels, which depolarizes the cell. Membrane depolarization and the increased $[Ca^{2+}]_i$ act together to open Px1 hemichannels, allowing transmitter secretion [97].

Are pannexins implemented in pathology?

Recent findings indicate that non-junctional hemichannels, both connexons and pannexons, can open in both physiological and pathological conditions [26,146,156]. Pannexin1 has been implicated in cell response to several pathological insults, including inflammation and tumorigenesis. Acute inflammation in response to bacterial surface antigens, endotoxins and other pro-inflammatory stimuli are known to be initiated by the cryopyrin-dependent release of interleukin-1β (IL-1β) [157]. Transcriptional activation of IL-1β is initiated by diverse pro-inflammatory stimuli, many of which converge on stimulating the TLR (TOLL-like) receptor family, which triggers signaling cascades down to turn on the gene expression. Proteolytic procession and release of the mature 18 kDa form of IL-1β by macrophages is critically dependent on cryopyrin-induced activation of caspase-1. These initial steps of inflammatory response, the activation of 'inflammasome' complex, involve three key steps: 1) the entry of the pro-inflammatory bacterial molecules into the cytosol, 2) proteolytic activation of caspase-1 by cryopyrin imflammasome and 3) cleavage of pro-IL-1β and release of mature IL-1β into the extracellular space [158,159]. Step 1 is facilitated by a secondary stimulus, the exogenous ATP pulse being the most potent of them [158-160]. Application of ATP on macrophage cells activates P2X7 receptor (P2X7R), a cation-selective ion channel [161]. P2X7R is commonly known as an unusual channel, which, upon stimulation by extracellular ATP evokes both ionic current and opening of a non-selective pore capable of passing molecules up to 900 Da (fluorescent dyes ethidium and YoPro1) [161,162]. A large pore opening can facilitate entry of pro-inflammatory molecules and was investigated in a series of studies that utilized an anti-human *PANX1* siRNA to specifically inhibit this gene, as well as specific mimetic peptide to block PANX1 protein function in cultured cells [23-25]. They demonstrated that endogenous *PANX1* expressed by macrophages represents such a pore in the initiation of Step 1 leading to caspase-1 activation. Pannexin1 hemichannel opening by ATP-stimulated P2X7R was required for caspase-1 cleavage and subsequent IL-1β maturation/release evoked by a spectrum of stimuli: the marine toxin (maitotoxin) and the K^+/H^+ antiport ionophore nigericin [163-165], and by bacterial proteins and toxins [23] . Blockade of the pannexin1 channels with mimetic peptide in macrophages expressing *P2X7R* prevented large pore formation and initial dye uptake, as well as processing and release IL-1β in response to P2X7R activation. However, this blockade failed to suppress P2X7R-specific ionic current and slow dye uptake. These studies demonstrated that pannexin1 hemichannel opening, induced by ATP stimulation of purinergic receptors, represents the entry pathway for bacterial inflammatory proteins to the cytosol, where they facilitate the activation of cryopyrin-dependent inflammasome and caspase-1 [23]. These studies emphasize that PANX1 is the critical and required component of the acute inflammatory response (Fig. 2). Importantly, parallel upregulation of the *P2X7R* and *PANX1* genes in response to phorbol esters has been detected in macrophages [166]. Typically, $P2X_7Rs$ expression in macropaphages is known to be regulated by pro-inflammatory stimuli such as interferon-gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), suggesting a possibility of parallel regulation of *PANX1* as part of the synergy in the inflammatory response. Consistent with this hypothesis, our search in the GEO (http://www.ncbi.nlm.nih.gov/projects/geo/) database revealed several independent microarray data sets showing a 3–7-fold elevation in the *PANX1* transcript level in experiments analyzing the effects of various pro-inflammatory stimuli: TNF-α (GEO## *GDS1543; GDS852; GDS1542*), IFN-α and IFN-γ (*GDS776; GDS1036*), lipopolysaccharide (LPS, in *GDS1249*), cold (*GDS1276*) and systemic inflammation (*GDS1276*) (Fig. 4). Similar responses were detected in multiple mammalian cell lines and tissues: human monocyte-derived dendritic cells (MDDCs), micro-

glia, microvascular umbilical vein endothelial cells (HUVECs), human microvascular endothelial cells (HMECs), alveolar epithelial cells, in adrenal medulla tissue (AM), as well as the inflammatory injury of whole lung and skeletal muscles tissues. Striking similarity in changes of the *PANX1* expression upon exposure to diverse pro-inflammatory stimuli supports the hypothesis that this protein is indeed an integral component of the inflammatory response at the cellular level (Fig. 2).

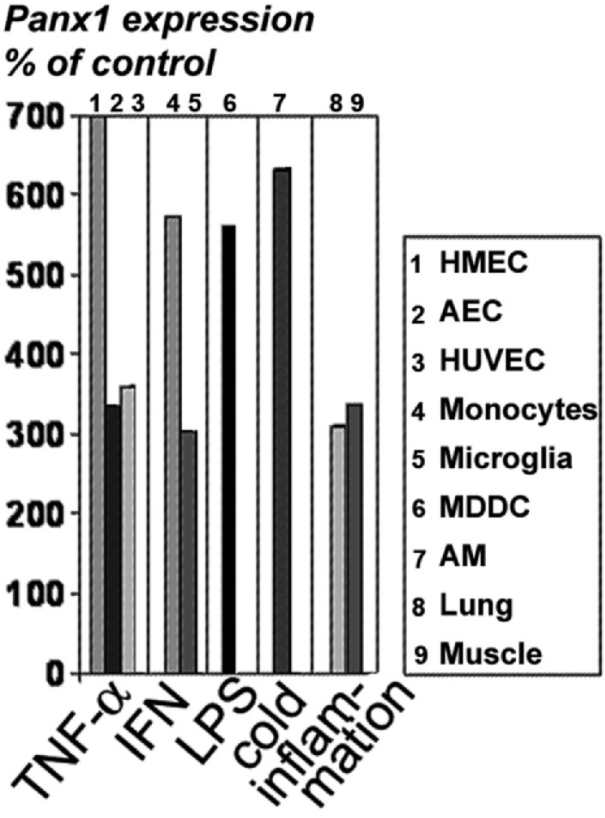

Figure 4. Significant increase in PANX1 expression in response to pro-inflammatory stimuli has been revealed by the analysis of gene expression data from multiple mammalian cell lines and tissues. Expression levels are shown as percentage of the control. GEO datasets used: *1*) GDS1543; *2*) GDS852; *3*) GDS1542; *4*) GDS776; *5*) GDS1036; *6*) GDS124; *7*) GDS 1851; *8*) GDS1276; *9*) GDS1259. HUVEC, human microvascular umbilical vein endothelial cells; HMEC, human microvascular endothelial cells; AEC, alveolar epithelium cells; MDDC, monocyte-derived dendritic cells; AM, adrenal medulla. Statistical significance was not calculated for all datasets presented in GEO.

Functional hemichannels (Cx43 connexons) were previously reported to play a role in cell damage under pathological conditions [167]. Neuronal excitotoxicity during stroke is caused by activation of unidentified large-conductance channels, leading to swelling, calcium dysregulation and necrotic cellular death [26]. The opening of large numbers of hemichannels following ischemia or inflammatory injury has also been suggested as a trigger for the pathophysiological cascade leading to cell depolarization, collapse of ionic gradients, loss of small metabolites and elevation of intracellular calcium. Hemichannel opening is typically detected by selective low molecular dye uptake: challenged cells become permeable to calcein (M_r 660), but not to dextran-conjugated fluorescein (M_r 1500–3000). In ischemic HEK-293 cells overexpressing exogenous *Cx43*, pore opening was activated by low extracellular calcium and blocked by lanthanum, properties similar to those of Cx43. In pyramidal neurons that do not express connexins but express endogenous Panx1, ischemia/glucose deprivation injury also caused large (550 pS) currents resulting in neuronal swelling, permeability to 660 Da calcein green dye and membrane breakdown, the effects of which can be blocked by hemichannel inhibitors CBX or lanthanum [26]. Physiological characteristics of the membrane pores causing ischemic death in acutely isolated pyramidal neurons allowed the authors to attribute a pathological mechanism to the activity of the endogenous Panx1 hemichannels.

Opening of hemichannels following metabolic inhibition has also been reported in primary cultures of rat and mouse astrocytes [98]. The current consensus on the mechanism by which metabolic inhibition triggers the opening of hemichannels implies dephosphorylation-induced dilation of Cx43 hemichannels [99]. Multiple phosphorylation sites are also predicted for the Panx2 protein, which can form heteromeric (Panx1/Panx2) channels with distinct properties [57]. Although it is tempting to suggest pannexons for the role in metabolic inhibition, it remains to be tested whether the Panx1/Panx2 channels do open in such conditions.

Conclusions

Pannexins are a novel family of ubiquitous pan-metazoan proteins structurally similar to strictly vertebrate connexins but evolutionarily distinct from them. Similarities between the two families extend to the domain structure of monomers and the ability to form functional transmembrane channels, including full GJs spanning the plasma membrane of neighboring cells. Phylogenetic reconstruction revealed several phyla lacking both families, thus suggesting that undiscovered GJ families may still exist. Physiological studies revealed that basic pannexin channel properties are distinct from those of connexins and are more suited for transmembrane transport of Ca^{2+} and ATP in response to physiological and pathological stimuli.

The significance of pannexins for normal cell physiology, communication and responses to pathological conditions requires knockout data and remains to be further elucidated.

Acknowledgements. The authors thank Thomas White, Steven Roper, Vladimir Aleshin and John Rash for critical reading and discussion of this manuscript, and David Barakat and Julia Shestopalov for their help with text and illustrations. The Map Editor software (GeneGo Inc., St. Joseph, MI) was utilized for drawing some of the illustrations. The work was supported by NIH grants R01-EY14232 (V.S.), Center grant P30-EY014801 (BPEI) and RFBR grant 05-04-48401 (Y.P.).

1 White, J.M. (1992) Membrane fusion. Science. 258, 917-24.
2 Shestopalov, V.I. and Bassnett, S. (2003) Development of a macromolecular diffusion pathway in the lens. J Cell Sci. 116, 4191-9.
3 Lubarsky, B. and Krasnow, M.A. (2003) Tube morphogenesis: making and shaping biological tubes. Cell. 112, 19-28.
4 Fleissner, A., Sarkar, S., Jacobson, D.J., Roca, M.G., Read, N.D. and Glass, N.L. (2005) The so locus is required for vegetative cell fusion and postfertilization events in Neurospora crassa. Eukaryot Cell. 4, 920-30.
5 Kaji, K. and Kudo, A. (2004) The mechanism of sperm-oocyte fusion in mammals. Reproduction. 127, 423-9.
6 Laird, D.W. (2006) Life cycle of connexins in health and disease. Biochem J. 394, 527-43.
7 Sohal, V.S. and Huguenard, J.R. (2005) Inhibitory coupling specifically generates emergent gamma oscillations in diverse cell types. Proc Natl Acad Sci U S A. 102, 18638-43.
8 Rozental, R., Carvalho, A.C. and Spray, D.C. (2000) Gap junctions in the cardiovascular and immune systems. Braz J Med Biol Res. 33, 365-8.
9 White, T.W. and Paul, D.L. (1999) Genetic diseases and gene knockouts reveal diverse connexin functions. Annu Rev Physiol. 61, 283-310.
10 Lo, C.W. (1999) Genes, gene knockouts, and mutations in the analysis of gap junctions. Dev Genet. 24, 1-4.
11 Simon, A.M. and Goodenough, D.A. (1998) Diverse functions of vertebrate gap junctions. Trends Cell Biol. 8, 477-83.
12 Bruzzone, R., White, T.W. and Goodenough, D.A. (1996) The cellular Internet: on-line with connexins. Bioessays. 18, 709-18.
13 Larsen, W.J. and Wert, S.E. (1988) Roles of cell junctions in gametogenesis and in early embryonic development. Tissue Cell. 20, 809-48.
14 Trexler, E.B., Bennett, M.V., Bargiello, T.A. and Verselis, V.K. (1996) Voltage gating and permeation in a gap junction hemichannel. Proc Natl Acad Sci U S A. 93, 5836-41.
15 Saez, J.C., Retamal, M.A., Basilio, D., Bukauskas, F.F. and Bennett, M.V. (2005) Connexin-based gap junction hemichannels: gating mechanisms. Biochim Biophys Acta. 1711, 215-24.
16 Goodenough, D.A. (1974) Bulk isolation of mouse hepatocyte gap junctions. Characterization of the principal protein, connexin. J Cell Biol. 61, 557-63.
17 Paul, D.L. (1986) Molecular cloning of cDNA for rat liver gap junction protein. J Cell Biol. 103, 123-34.
18 Baranova, A., Ivanov, D., Petrash, N., Pestova, A., Skoblov, M., Kelmanson, I., Shagin, D., Nazarenko, S., Geraymovych, E., Litvin, O., Tiunova, A., Born, T.L., Usman, N., Staroverov, D., Lukyanov, S. and Panchin, Y. (2004) The mammalian pannexin family is homologous to the invertebrate innexin gap junction proteins. Genomics. 83, 706-16.
19 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, R473-4.
20 Zampighi, G.A., Hall, J.E., Ehring, G.R. and Simon, S.A. (1989) The structural organization and protein composition of lens fiber junctions. J Cell Biol. 108, 2255-75.
21 Goodenough, D.A. and Paul, D.L. (2003) Beyond the gap: functions of unpaired connexon channels. Nat Rev Mol Cell Biol. 4, 285-94.
22 Falk, M.M. (2000) Biosynthesis and structural composition of gap junction intercellular membrane channels. Eur J Cell Biol. 79, 564-74.
23 Kanneganti, T.D., Lamkanfi, M., Kim, Y.G., Chen, G., Park, J.H., Franchi, L., Vandenabeele, P. and Nunez, G. (2007) Pannexin-1-mediated recognition of bacterial molecules activates the cryopyrin inflammasome independent of Toll-like receptor signaling. Immunity. 26, 433-43.
24 Pelegrin, P. and Surprenant, A. (2007) Pannexin-1 couples to maitotoxin- and nigericin-induced interleukin-1beta release through a dye uptake-independent pathway. J Biol Chem. 282, 2386-94.
25 Pelegrin, P. and Surprenant, A. (2006) Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor. Embo J. 25, 5071-82.
26 Thompson, R.J., Zhou, N. and MacVicar, B.A. (2006) Ischemia opens neuronal gap junction hemichannels. Science. 312, 924-7.
27 Lai, C.P., Bechberger, J.F., Thompson, R.J., Macvicar, B.A., Bruzzone, R. and Naus, C.C. (2007) Tumor-Suppressive Effects of Pannexin 1 in C6 Glioma Cells. Cancer Res. 67, 1545-1554.
28 Bennett, M.V. and Goodenough, D.A. (1978) Gap junctions, electrotonic coupling, and intercellular communication. Neurosci Res Program Bull. 16, 1-486.
29 White, T.W. (2002) Unique and redundant connexin contributions to lens development. Science. 295, 319-20.
30 Goodenough, D.A., Dick, J.S., 2nd and Lyons, J.E. (1980) Lens metabolic cooperation: a study of mouse lens transport and permeability visualized with freeze-substitution autoradiography and electron microscopy. J Cell Biol. 86, 576-89.
31 Goodenough, D.A. (1979) Lens gap junctions: a structural hypothesis for nonregulated low- resistance intercellular pathways. Invest Ophthalmol Vis Sci. 18, 1104-22.
32 Kihara, A.H., Mantovani de Castro, L., Belmonte, M.A., Yan, C.Y., Moriscot, A.S. and Hamassaki, D.E. (2006) Expression of connexins 36, 43, and 45 during postnatal development of the mouse retina. J Neurobiol. 66, 1397-410.
33 Feigenspan, A., Teubner, B., Willecke, K. and Weiler, R. (2001) Expression of neuronal connexin36 in AII amacrine cells of the mammalian retina. J Neurosci. 21, 230-9.
34 Curtin, K.D., Zhang, Z. and Wyman, R.J. (2002) Gap junction proteins are not interchangeable in development of neural function in the Drosophila visual system. J Cell Sci. 115, 3379-88.
35 Wallraff, A., Odermatt, B., Willecke, K. and Steinhauser, C. (2004) Distinct types of astroglial cells in the hippocampus differ in gap junction coupling. Glia. 48, 36-43.
36 Meier, C. and Dermietzel, R. (2006) Electrical synapses–gap junctions in the brain. Results Probl Cell Differ. 43, 99-128.
37 Fukuda, T., Kosaka, T., Singer, W. and Galuske, R.A. (2006) Gap junctions among dendrites of cortical GABAergic neurons establish a dense and widespread intercolumnar network. J Neurosci. 26, 3434-43.
38 Sutor, B. (2002) Gap junctions and their implications for neurogenesis and maturation of synaptic circuitry in the developing neocortex. Results Probl Cell Differ. 39, 53-73.
39 Goodenough, D.A. (1992) The crystalline lens. A system networked by gap junctional intercellular communication. Semin Cell Biol. 3, 49-58.
40 van Kempen, M., van Ginneken, A., de Grijs, I., Mutsaers, N., Opthof, T., Jongsma, H. and van der Heyden, M. (2003) Expression of the electrophysiological system during murine embryonic stem cell cardiac differentiation. Cell Physiol Biochem. 13, 263-70.

41 Westfall, M.V., Pasyk, K.A., Yule, D.I., Samuelson, L.C. and Metzger, J.M. (1997) Ultrastructure and cell-cell coupling of cardiac myocytes differentiating in embryonic stem cell cultures. Cell Motil Cytoskeleton. 36, 43-54.
42 Goodenough, D.A., Simon, A.M. and Paul, D.L. (1999) Gap junctional intercellular communication in the mouse ovarian follicle. Novartis Found Symp. 219, 226-35; discussion 235-40.
43 Wiesen, J.F. and Midgley, A.R., Jr. (1993) Changes in expression of connexin 43 gap junction messenger ribonucleic acid and protein during ovarian follicular growth. Endocrinology. 133, 741-6.
44 Elenes, S., Martinez, A.D., Delmar, M., Beyer, E.C. and Moreno, A.P. (2001) Heterotypic docking of Cx43 and Cx45 connexons blocks fast voltage gating of Cx43. Biophys J. 81, 1406-18.
45 Rubin, J.B., Verselis, V.K., Bennett, M.V. and Bargiello, T.A. (1992) Molecular analysis of voltage dependence of heterotypic gap junctions formed by connexins 26 and 32. Biophys J. 62, 183-93; discussion 193-5.
46 Harris, A.L. and Bevans, C.G. (2001) Exploring hemichannel permeability in vitro. Methods Mol Biol. 154, 357-77.
47 Pfahnl, A. and Dahl, G. (1998) Localization of a voltage gate in connexin46 gap junction hemichannels. Biophys J. 75, 2323-31.
48 Gupta, V.K., Berthoud, V.M., Atal, N., Jarillo, J.A., Barrio, L.C. and Beyer, E.C. (1994) Bovine connexin44, a lens gap junction protein: molecular cloning, immunologic characterization, and functional expression. Invest Ophthalmol Vis Sci. 35, 3747-58.
49 Ebihara, L. and Steiner, E. (1993) Properties of a nonjunctional current expressed from a rat connexin46 cDNA in Xenopus oocytes. J Gen Physiol. 102, 59-74.
50 Quist, A.P., Rhee, S.K., Lin, H. and Lal, R. (2000) Physiological role of gap-junctional hemichannels. Extracellular calcium-dependent isosmotic volume regulation. J Cell Biol. 148, 1063-74.
51 Stout, C., Goodenough, D.A. and Paul, D.L. (2004) Connexins: functions without junctions. Curr Opin Cell Biol. 16, 507-12.
52 Chaytor, A.T., Martin, P.E., Evans, W.H., Randall, M.D. and Griffith, T.M. (1999) The endothelial component of cannabinoid-induced relaxation in rabbit mesenteric artery depends on gap junctional communication. J Physiol. 520 Pt 2, 539-50.
53 De Vuyst, E., Decrock, E., Cabooter, L., Dubyak, G.R., Naus, C.C., Evans, W.H. and Leybaert, L. (2006) Intracellular calcium changes trigger connexin 32 hemichannel opening. Embo J. 25, 34-44.
54 Dykes, I.M., Freeman, F.M., Bacon, J.P. and Davies, J.A. (2004) Molecular basis of gap junctional communication in the CNS of the leech Hirudo medicinalis. J Neurosci. 24, 886-94.
55 Dahl, G. and Locovei, S. (2006) Pannexin: to gap or not to gap, is that a question? IUBMB Life. 58, 409-19.
56 Bruzzone, R., Hormuzdi, S.G., Barbe, M.T., Herb, A. and Monyer, H. (2003) Pannexins, a family of gap junction proteins expressed in brain. Proc Natl Acad Sci U S A. 100, 13644-9.
57 Bruzzone, R., Barbe, M.T., Jakob, N.J. and Monyer, H. (2005) Pharmacological properties of homomeric and heteromeric pannexin hemichannels expressed in Xenopus oocytes. J Neurochem. 92, 1033-43.
58 Spray, D.C., Ye, Z.C. and Ransom, B.R. (2006) Functional connexin "hemichannels": a critical appraisal. Glia. 54, 758-73.
59 Locovei, S., Wang, J. and Dahl, G. (2006) Activation of pannexin 1 channels by ATP through P2Y receptors and by cytoplasmic calcium. FEBS Lett. 580, 239-44.
60 Bao, L., Locovei, S. and Dahl, G. (2004) in: FEBS Lett, Vol. 572, pp. 65-8.
61 Locovei, S., Bao, L. and Dahl, G. (2006) Pannexin 1 in erythrocytes: function without a gap. Proc Natl Acad Sci U S A. 103, 7655-9.
62 Vanden Abeele, F., Bidaux, G., Gordienko, D., Beck, B., Panchin, Y.V., Baranova, A.V., Ivanov, D.V., Skryma, R. and Prevarskaya, N. (2006) Functional implications of calcium permeability of the channel formed by pannexin 1. J Cell Biol. 174, 535-46.
63 Verselis, V.K. and Bukauskas, F.F. (2002) Connexin-GFPs shed light on regulation of cell-cell communication by gap junctions. Curr Drug Targets. 3, 483-99.
64 Huang, Y., Grinspan, J.B., Abrams, C.K. and Scherer, S.S. (2007) Pannexin1 is expressed by neurons and glia but does not form functional gap junctions. Glia. 55, 46-56.
65 Bauer, R., Loer, B., Ostrowski, K., Martini, J., Weimbs, A., Lechner, H. and Hoch, M. (2005) Intercellular communication: the Drosophila innexin multiprotein family of gap junction proteins. Chem Biol. 12, 515-26.
66 Phelan, P. and Starich, T.A. (2001) Innexins get into the gap. Bioessays. 23, 388-96.
67 Phelan, P. (2005) Innexins: members of an evolutionarily conserved family of gap-junction proteins. Biochim Biophys Acta. 1711, 225-45.
68 Panchin, Y.V. (2005) Evolution of gap junction proteins–the pannexin alternative. J Exp Biol. 208, 1415-9.
69 Litvin, O., Tiunova, A., Connell-Alberts, Y., Panchin, Y. and Baranova, A. (2006) What is hidden in the pannexin treasure trove: the sneak peek and the guesswork. J Cell Mol Med. 10, 613-34.
70 Barnes, T.M. (1994) OPUS: a growing family of gap junction proteins? Trends Genet. 10, 303-5.
71 Phelan, P., Bacon, J.P., Davies, J.A., Stebbings, L.A., Todman, M.G., Avery, L., Baines, R.A., Barnes, T.M., Ford, C., Hekimi, S., Lee, R., Shaw, J.E., Starich, T.A., Curtin, K.D., Sun, Y.A. and Wyman, R.J. (1998) Innexins: a family of invertebrate gap-junction proteins. Trends Genet. 14, 348-9.
72 Baldauf, S.L. (2003) The deep roots of eukaryotes. Science. 300, 1703-6.
73 Ducret, E., Alexopoulos, H., Le Feuvre, Y., Davies, J.A., Meyrand, P., Bacon, J.P. and Fenelon, V.S. (2006) Innexins in the lobster stomatogastric nervous system: cloning, phylogenetic analysis, developmental changes and expression within adult identified dye and electrically coupled neurons. Eur J Neurosci. 24, 3119-33.
74 Dykes, I.M. and Macagno, E.R. (2006) Molecular characterization and embryonic expression of innexins in the leech Hirudo medicinalis. Dev Genes Evol. 216, 185-97.
75 Alexopoulos, H., Bottger, A., Fischer, S., Levin, A., Wolf, A., Fujisawa, T., Hayakawa, S., Gojobori, T., Davies, J.A., David, C.N. and Bacon, J.P. (2004) Evolution of gap junctions: the missing link? Curr Biol. 14, R879-80.
76 White, T.W., Wang, H., Mui, R., Litteral, J. and Brink, P.R. (2004) Cloning and functional expression of invertebrate connexins from Halocynthia pyriformis. FEBS Lett. 577, 42-8.
77 Sasakura, Y., Shoguchi, E., Takatori, N., Wada, S., Meinertzhagen, I.A., Satou, Y. and Satoh, N. (2003) A genomewide survey of developmentally relevant genes in Ciona intestinalis. X. Genes for cell junctions and extracellular matrix. Dev Genes Evol. 213, 303-13.
78 Burke, R.D., Angerer, L.M., Elphick, M.R., Humphrey, G.W., Yaguchi, S., Kiyama, T., Liang, S., Mu, X., Agca, C., Klein, W.H., Brandhorst, B.P., Rowe, M., Wilson, K., Churcher, A.M., Taylor, J.S., Chen, N., Murray, G., Wang, D., Mellott, D., Olinski, R., Hallbook, F. and Thorndyke, M.C. (2006) A genomic view of the sea urchin nervous system. Dev Biol. 300, 434-60.
79 Sodergren, E., Weinstock, G.M., Davidson, E.H., Cameron, R.A., Gibbs, R.A., Angerer, R.C., Angerer, L.M., Arnone, M.I., Burgess, D.R., Burke, R.D., Coffman, J.A., Dean, M., Elphick, M.R., Ettensohn, C.A., Foltz, K.R., Hamdoun, A., Hynes, R.O., Klein, W.H., Marzluff, W. et al. (2006) The genome of the sea urchin Strongylocentrotus purpuratus. Science. 314, 941-52.
80 Bourlat, S.J., Juliusdottir, T., Lowe, C.J., Freeman, R., Aronowicz, J., Kirschner, M., Lander, E.S., Thorndyke, M.,

Nakano, H., Kohn, A.B., Heyland, A., Moroz, L.L., Copley, R.R. and Telford, M.J. (2006) Deuterostome phylogeny reveals monophyletic chordates and the new phylum Xenoturbellida. Nature. 444, 85-8.
81 Delsuc, F., Brinkmann, H., Chourrout, D. and Philippe, H. (2006) Tunicates and not cephalochordates are the closest living relatives of vertebrates. Nature. 439, 965-8.
82 Yazaki, I., Dale, B. and Tosti, E. (1999) Functional gap junctions in the early sea urchin embryo are localized to the vegetal pole. Dev Biol. 212, 503-10.
83 Mire, P., Nasse, J. and Venable-Thibodeaux, S. (2000) Gap junctional communication in the vibration-sensitive response of sea anemones. Hear Res. 144, 109-23.
84 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, 1176-80.
85 Unger, V.M., Kumar, N.M., Gilula, N.B. and Yeager, M. (1997) Projection structure of a gap junction membrane channel at 7 A resolution. Nat Struct Biol. 4, 39-43.
86 Makowski, L., Caspar, D.L., Phillips, W.C., Baker, T.S. and Goodenough, D.A. (1984) Gap junction structures. VI. Variation and conservation in connexon conformation and packing. Biophys J. 45, 208-18.
87 Barbe, M.T., Monyer, H. and Bruzzone, R. (2006) Cell-cell communication beyond connexins: the pannexin channels. Physiology (Bethesda). 21, 103-14.
88 Peracchia, C. (1973) Low resistance junctions in crayfish. II. Structural details and further evidence for intercellular channels by freeze-fracture and negative staining. J Cell Biol. 57, 54-65.
89 Peracchia, C. (1973) Low resistance junctions in crayfish. I. Two arrays of globules in junctional membranes. J Cell Biol. 57, 66-76.
90 Rash, J.E., Yasumura, T. and Dudek, F.E. (1998) Ultrastructure, histological distribution, and freeze-fracture immunocytochemistry of gap junctions in rat brain and spinal cord. Cell Biol Int. 22, 731-49.
91 Baldo, G.J., Gong, X., Martinez-Wittinghan, F.J., Kumar, N.M., Gilula, N.B. and Mathias, R.T. (2001) Gap junctional coupling in lenses from alpha(8) connexin knockout mice. J Gen Physiol. 118, 447-56.
92 Gong, X., Baldo, G.J., Kumar, N.M., Gilula, N.B. and Mathias, R.T. (1998) Gap junctional coupling in lenses lacking alpha3 connexin. Proc Natl Acad Sci U S A. 95, 15303-8.
93 Dvoriantchikova, G., Ivanov, D., Pestova, A. and Shestopalov, V. (2006) Molecular characterization of pannexins in the lens. Mol Vis. 12, 1417-26.
94 Ebihara, L. (2003) New roles for connexons. News Physiol Sci. 18, 100-3.
95 Castro, C., Gomez-Hernandez, J.M., Silander, K. and Barrio, L.C. (1999) Altered formation of hemichannels and gap junction channels caused by C-terminal connexin-32 mutations. J Neurosci. 19, 3752-60.
96 Romanov, R.A., Rogachevskaja, O.A., Bystrova, M.F., Jiang, P., Margolskee, R.F. and Kolesnikov, S.S. (2007) Afferent neurotransmission mediated by hemichannels in mammalian taste cells. Embo J. 26, 657-67.
97 Huang, Y.J., Maruyama, Y., Dvoryanchikov, G., Pereira, E., Chaudhari, N. and Roper, S.D. (2007) The role of pannexin 1 hemichannels in ATP release and cell-cell communication in mouse taste buds. Proc Natl Acad Sci U S A.
98 Contreras, J.E., Sanchez, H.A., Eugenin, E.A., Speidel, D., Theis, M., Willecke, K., Bukauskas, F.F., Bennett, M.V. and Saez, J.C. (2002) Metabolic inhibition induces opening of unapposed connexin 43 gap junction hemichannels and reduces gap junctional communication in cortical astrocytes in culture. Proc Natl Acad Sci U S A. 99, 495-500.
99 Kim, D.Y., Kam, Y., Koo, S.K. and Joe, C.O. (1999) Gating connexin 43 channels reconstituted in lipid vesicles by mitogen-activated protein kinase phosphorylation. J Biol Chem. 274, 5581-7.
100 Moreno, A.P. (2005) Connexin phosphorylation as a regulatory event linked to channel gating. Biochim Biophys Acta. 1711, 164-71.
101 Vogt, A., Hormuzdi, S.G. and Monyer, H. (2005) Pannexin1 and Pannexin2 expression in the developing and mature rat brain. Brain Res Mol Brain Res. 141, 113-20.
102 Ray, A., Zoidl, G., Weickert, S., Wahle, P. and Dermietzel, R. (2005) Site-specific and developmental expression of pannexin1 in the mouse nervous system. Eur J Neurosci. 21, 3277-90.
103 Dvoriantchikova, G., Ivanov, D., Panchin, Y. and Shestopalov, V.I. (2006) Expression of pannexin family of proteins in the retina. FEBS Lett. 580, 2178-82.
104 Ray, A., Zoidl, G., Wahle, P. and Dermietzel, R. (2006) Pannexin expression in the cerebellum. Cerebellum. 5, 189-92.
105 Cotrina, M.L., Lin, J.H., Alves-Rodrigues, A., Liu, S., Li, J., Azmi-Ghadimi, H., Kang, J., Naus, C.C. and Nedergaard, M. (1998) Connexins regulate calcium signaling by controlling ATP release. Proc Natl Acad Sci U S A. 95, 15735-40.
106 Cotrina, M.L., Lin, J.H., Lopez-Garcia, J.C., Naus, C.C. and Nedergaard, M. (2000) ATP-mediated glia signaling. J Neurosci. 20, 2835-44.
107 Jorgensen, N.R., Henriksen, Z., Brot, C., Eriksen, E.F., Sorensen, O.H., Civitelli, R. and Steinberg, T.H. (2000) Human osteoblastic cells propagate intercellular calcium signals by two different mechanisms. J Bone Miner Res. 15, 1024-32.
108 Stout, C.E., Costantin, J.L., Naus, C.C. and Charles, A.C. (2002) Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels. J Biol Chem. 277, 10482-8.
109 White, T.W., Deans, M.R., O'Brien, J., Al-Ubaidi, M.R., Goodenough, D.A., Ripps, H. and Bruzzone, R. (1999) Functional characteristics of skate connexin35, a member of the gamma subfamily of connexins expressed in the vertebrate retina. Eur J Neurosci. 11, 1883-90.
110 Evans, W.H. and Martin, P.E. (2002) Lighting up gap junction channels in a flash. Bioessays. 24, 876-80.
111 Bukauskas, F.F., Kreuzberg, M.M., Rackauskas, M., Bukauskiene, A., Bennett, M.V., Verselis, V.K. and Willecke, K. (2006) Properties of mouse connexin 30.2 and human connexin 31.9 hemichannels: implications for atrioventricular conduction in the heart. Proc Natl Acad Sci U S A. 103, 9726-31.
112 Peracchia, C. (2004) Chemical gating of gap junction channels; roles of calcium, pH and calmodulin. Biochim Biophys Acta. 1662, 61-80.
113 Pfahnl, A. and Dahl, G. (1999) Gating of cx46 gap junction hemichannels by calcium and voltage. Pflugers Arch. 437, 345-53.
114 Scemes, E., Dermietzel, R. and Spray, D.C. (1998) Calcium waves between astrocytes from Cx43 knockout mice. Glia. 24, 65-73.
115 Zappala, A., Cicero, D., Serapide, M.F., Paz, C., Catania, M.V., Falchi, M., Parenti, R., Panto, M.R., La Delia, F. and Cicirata, F. (2006) Expression of pannexin1 in the CNS of adult mouse: cellular localization and effect of 4-aminopyridine-induced seizures. Neuroscience. 141, 167-78.
116 Crow, J.M., Atkinson, M.M. and Johnson, R.G. (1994) Micromolar levels of intracellular calcium reduce gap junctional permeability in lens cultures. Invest Ophthalmol Vis Sci. 35, 3332-41.
117 Lazrak, A. and Peracchia, C. (1993) Gap junction gating sensitivity to physiological internal calcium regardless of pH in Novikoff hepatoma cells. Biophys J. 65, 2002-12.
118 Sanderson, M.J., Charles, A.C., Boitano, S. and Dirksen, E.R. (1994) Mechanisms and function of intercellular calcium signaling. Mol Cell Endocrinol. 98, 173-87.
119 Boitano, S., Dirksen, E.R. and Sanderson, M.J. (1992) Intercellular propagation of calcium waves mediated by inositol trisphosphate. Science. 258, 292-5.

120 Saez, J.C., Connor, J.A., Spray, D.C. and Bennett, M.V. (1989) Hepatocyte gap junctions are permeable to the second messenger, inositol 1,4,5-trisphosphate, and to calcium ions. Proc Natl Acad Sci U S A. 86, 2708-12.
121 Osipchuk, Y. and Cahalan, M. (1992) Cell-to-cell spread of calcium signals mediated by ATP receptors in mast cells. Nature. 359, 241-4.
122 Gallagher, C.J. and Salter, M.W. (2003) Differential properties of astrocyte calcium waves mediated by P2Y1 and P2Y2 receptors. J Neurosci. 23, 6728-39.
123 Di Virgilio, F., Chiozzi, P., Ferrari, D., Falzoni, S., Sanz, J.M., Morelli, A., Torboli, M., Bolognesi, G. and Baricordi, O.R. (2001) Nucleotide receptors: an emerging family of regulatory molecules in blood cells. Blood. 97, 587-600.
124 Harrington, L.S. and Mitchell, J.A. (2004) Novel role for P2X receptor activation in endothelium-dependent vasodilation. Br J Pharmacol. 143, 611-7.
125 Dietrich, H.H., Ellsworth, M.L., Sprague, R.S. and Dacey, R.G., Jr. (2000) Red blood cell regulation of microvascular tone through adenosine triphosphate. Am J Physiol Heart Circ Physiol. 278, H1294-8.
126 Bergfeld, G.R. and Forrester, T. (1992) Release of ATP from human erythrocytes in response to a brief period of hypoxia and hypercapnia. Cardiovasc Res. 26, 40-7.
127 Sprague, R.S., Ellsworth, M.L., Stephenson, A.H. and Lonigro, A.J. (1996) ATP: the red blood cell link to NO and local control of the pulmonary circulation. Am J Physiol. 271, H2717-22.
128 Weickert, S., Ray, A., Zoidl, G. and Dermietzel, R. (2005) Expression of neural connexins and pannexin1 in the hippocampus and inferior olive: a quantitative approach. Brain Res Mol Brain Res. 133, 102-9.
129 Sohl, G., Maxeiner, S. and Willecke, K. (2005) Expression and functions of neuronal gap junctions. Nat Rev Neurosci. 6, 191-200.
130 Paternostro, M.A., Reyher, C.K. and Brunjes, P.C. (1995) Intracellular injections of lucifer yellow into lightly fixed mitral cells reveal neuronal dye-coupling in the developing rat olfactory bulb. Brain Res Dev Brain Res. 84, 1-10.
131 Sutor, B. and Hagerty, T. (2005) Involvement of gap junctions in the development of the neocortex. Biochim Biophys Acta. 1719, 59-68.
132 Khazipov, R. and Luhmann, H.J. (2006) Early patterns of electrical activity in the developing cerebral cortex of humans and rodents. Trends Neurosci. 29, 414-8.
133 Le Van Quyen, M., Khalilov, I. and Ben-Ari, Y. (2006) The dark side of high-frequency oscillations in the developing brain. Trends Neurosci. 29, 419-27.
134 Moody, W.J. and Bosma, M.M. (2005) Ion channel development, spontaneous activity, and activity-dependent development in nerve and muscle cells. Physiol Rev. 85, 883-941.
135 Rakic, P. and Komuro, H. (1995) The role of receptor/channel activity in neuronal cell migration. J Neurobiol. 26, 299-315.
136 Pakhotin, P. and Verkhratsky, A. (2005) Electrical synapses between Bergmann glial cells and Purkinje neurones in rat cerebellar slices. Mol Cell Neurosci. 28, 79-84.
137 Deans, M.R., Gibson, J.R., Sellitto, C., Connors, B.W. and Paul, D.L. (2001) Synchronous activity of inhibitory networks in neocortex requires electrical synapses containing connexin36. Neuron. 31, 477-85.
138 Hormuzdi, S.G., Pais, I., LeBeau, F.E., Towers, S.K., Rozov, A., Buhl, E.H., Whittington, M.A. and Monyer, H. (2001) Impaired electrical signaling disrupts gamma frequency oscillations in connexin 36-deficient mice. Neuron. 31, 487-95.
139 Traub, R.D., Whittington, M.A., Buhl, E.H., LeBeau, F.E., Bibbig, A., Boyd, S., Cross, H. and Baldeweg, T. (2001) A possible role for gap junctions in generation of very fast EEG oscillations preceding the onset of, and perhaps initiating, seizures. Epilepsia. 42, 153-70.
140 Altevogt, B.M. and Paul, D.L. (2004) Four classes of intercellular channels between glial cells in the CNS. J Neurosci. 24, 4313-23.
141 Nagy, J.I. and Rash, J.E. (2000) Connexins and gap junctions of astrocytes and oligodendrocytes in the CNS. Brain Res Brain Res Rev. 32, 29-44.
142 Rash, J.E., Yasumura, T., Dudek, F.E. and Nagy, J.I. (2001) Cell-specific expression of connexins and evidence of restricted gap junctional coupling between glial cells and between neurons. J Neurosci. 21, 1983-2000.
143 He, L.Q., Cai, F., Liu, Y., Liu, M.J., Tan, Z.P., Pan, Q., Fang, F.Y., Liang de, S., Wu, L.Q., Long, Z.G., Dai, H.P., Xia, K., Xia, J.H. and Zhang, Z.H. (2005) Cx31 is assembled and trafficked to cell surface by ER-Golgi pathway and degraded by proteasomal or lysosomal pathways. Cell Res. 15, 455-64.
144 Martin, P.E., Blundell, G., Ahmad, S., Errington, R.J. and Evans, W.H. (2001) Multiple pathways in the trafficking and assembly of connexin 26, 32 and 43 into gap junction intercellular communication channels. J Cell Sci. 114, 3845-55.
145 Zoidl, G., Petrasch-Parwez, E., Ray, A., Meier, C., Bunse, S., Habbes, H.W., Dahl, G. and Dermietzel, R. (2007) Localization of the pannexin1 protein at postsynaptic sites in the cerebral cortex and hippocampus. Neuroscience. 146, 9-16.
146 Parpura, V., Scemes, E. and Spray, D.C. (2004) Mechanisms of glutamate release from astrocytes: gap junction "hemichannels", purinergic receptors and exocytotic release. Neurochem Int. 45, 259-64.
147 Di Iorio, P., Ballerini, P., Caciagli, F. and Ciccarelli, R. (1998) Purinoceptor-mediated modulation of purine and neurotransmitter release from nervous tissue. Pharmacol Res. 37, 169-78.
148 Charles, A.C., Naus, C.C., Zhu, D., Kidder, G.M., Dirksen, E.R. and Sanderson, M.J. (1992) Intercellular calcium signaling via gap junctions in glioma cells. J Cell Biol. 118, 195-201.
149 Wong, C.W., Christen, T., Roth, I., Chadjichristos, C.E., Derouette, J.P., Foglia, B.F., Chanson, M., Goodenough, D.A. and Kwak, B.R. (2006) Connexin37 protects against atherosclerosis by regulating monocyte adhesion. Nat Med. 12, 950-4.
150 E, L.L., Zhao, Y.S., Guo, X.M., Wang, C.Y., Jiang, H., Li, J., Duan, C.M. and Song, Y. (2006) Enrichment of cardiomyocytes derived from mouse embryonic stem cells. J Heart Lung Transplant. 25, 664-74.
151 Kamermans, M., Fahrenfort, I., Schultz, K., Janssen-Bienhold, U., Sjoerdsma, T. and Weiler, R. (2001) Hemichannel-mediated inhibition in the outer retina. Science. 292, 1178-80.
152 Hombach, S., Janssen-Bienhold, U., Sohl, G., Schubert, T., Bussow, H., Ott, T., Weiler, R. and Willecke, K. (2004) Functional expression of connexin57 in horizontal cells of the mouse retina. Eur J Neurosci. 19, 2633-40.
153 Shelley, J., Dedek, K., Schubert, T., Feigenspan, A., Schultz, K., Hombach, S., Willecke, K. and Weiler, R. (2006) Horizontal cell receptive fields are reduced in connexin57-deficient mice. Eur J Neurosci. 23, 3176-86.
154 Roper, S.D. (2006) Cell communication in taste buds. Cell Mol Life Sci. 63, 1494-500.
155 Romanov, R.A. and Kolesnikov, S.S. (2006) Electrophysiologically identified subpopulations of taste bud cells. Neurosci Lett. 395, 249-54.
156 Bennett, M.V., Contreras, J.E., Bukauskas, F.F. and Saez, J.C. (2003) New roles for astrocytes: gap junction hemichannels have something to communicate. Trends Neurosci. 26, 610-7.
157 Dinarello, C.A. (2005) Blocking IL-1 in systemic inflammation. J Exp Med. 201, 1355-9.
158 Rothwell, N.J. and Luheshi, G.N. (2000) Interleukin 1 in the brain: biology, pathology and therapeutic target. Trends Neurosci. 23, 618-25.
159 Basu, A., Krady, J.K. and Levison, S.W. (2004) Interleukin-1: a master regulator of neuroinflammation. J Neurosci Res. 78, 151-6.
160 Allan, S.M., Tyrrell, P.J. and Rothwell, N.J. (2005) Interleukin-1 and neuronal injury. Nat Rev Immunol. 5, 629-40.
161 Ferrari, D., Pizzirani, C., Adinolfi, E., Lemoli, R.M., Curti, A., Idzko, M., Panther, E. and Di Virgilio, F. (2006) The P2X7

receptor: a key player in IL-1 processing and release. J Immunol. 176, 3877-83.
162 North, R.A. (2002) Molecular physiology of P2X receptors. Physiol Rev. 82, 1013-67.
163 Schilling, W.P., Wasylyna, T., Dubyak, G.R., Humphreys, B.D. and Sinkins, W.G. (1999) Maitotoxin and P2Z/P2X(7) purinergic receptor stimulation activate a common cytolytic pore. Am J Physiol. 277, C766-76.
164 Warny, M. and Kelly, C.P. (1999) Monocytic cell necrosis is mediated by potassium depletion and caspase-like proteases. Am J Physiol. 276, C717-24.
165 Verhoef, P.A., Kertesy, S.B., Estacion, M., Schilling, W.P. and Dubyak, G.R. (2004) Maitotoxin induces biphasic interleukin-1beta secretion and membrane blebbing in murine macrophages. Mol Pharmacol. 66, 909-20.
166 Humphreys, B.D. and Dubyak, G.R. (1998) Modulation of P2X7 nucleotide receptor expression by pro- and anti-inflammatory stimuli in THP-1 monocytes. J Leukoc Biol. 64, 265-73.
167 John, S.A., Kondo, R., Wang, S.Y., Goldhaber, J.I. and Weiss, J.N. (1999) Connexin-43 hemichannels opened by metabolic inhibition. J Biol Chem. 274, 236-40.