The establishment of multicellularity has required the development of different forms of interactions to monitor and coordinate the activity of groups of cells. Specialized, yet distinct structures have emerged independently to provide direct cell-to-cell communication in plants (which use plasmodesmata), fungi (which use septal pores), and animals (which use gap junctions). The novel form of direct communication dependent on so-called tunneling nanotubes, which has been recently discovered in mammalian cells and correlated to the transfer of organelles from one cell to the other (78), will not be discussed here.

Gap junctions are the only junctional structures that are conserved in all multicellular organisms from mesozoa to humans, with few exceptions such as the slime mold Dictyostelium discoideum. Despite the maintenance of both a morphologically similar structure (the heptalaminar appearance and intercellular gap) and functional properties (permeability to ions and small molecules), genome analysis has revealed an interesting case of convergent evolution across the animal kingdom, with unrelated protein families having evolved to perform similar functions. Thus it has now been conclusively demonstrated that gap-junction channels spanning the plasma membranes of the two connected cells are multimeric complexes that result from the association of two hemichannels, or connexons, each composed of six connexin subunits. Such intercellular channels are ubiquitous, albeit with notable exceptions (e.g., spermatozoa, red blood cells, and adult skeletal myocytes), and further

Molecular Identity of Gap-Junction Proteins

Connexins are a family of proteins that have been investigated at the molecular level during the last two decades. Since the cloning of the first members at the end of the 1980s, the family has considerably expanded, and there are now 21 genes in the human and 20 in the mouse genome (109). Connexins (abbreviated Cx with the molecular mass in kDa appended: Cx32, Cx43, etc.) appear to be chordate specific, since no connexin homolog has been identified in the genomes of nonchordate metazoans, such as worm or fly. Connexin channels spanning the plasma membranes of the two connected cells are multimeric complexes that result from the association of two hemichannels, or connexons, each composed of six connexin subunits. Such intercellular channels are ubiquitous, albeit with notable exceptions (e.g., spermatozoa, red blood cells, and adult skeletal myocytes), and further
more, recent studies have revealed that unpaired connexons in the nonjunctional plasma membrane can also be active as hemichannels that bridge the cytoplasm with the extracellular space (36, 47, 53). Although the role of hemichannels in vivo remains to be determined, this property could potentially expand the functional range in which connexins are implicated. Most connexins have been studied at the molecular level, and their properties in terms of channel assembly (homomeric and heteromeric) permeability, size and ionic selectivity, compatibility (homotypic and heterotypic), and gating mechanisms have been investigated in great detail (see Refs. 17, 38, 45, 49, 62, 67, 79, 92, 94, 98 for further reading and reference to primary publications). Although many aspects have not yet been elucidated, these studies have allowed the emergence of a different appreciation of connexin channels, which are no longer viewed as mere passive pores for the free transfer of ions and molecules below a fixed molecular mass but are now believed to fulfill specific functions. Proof of this concept has been obtained by replacing one connexin gene with another via genetic knock-in, thereby providing a direct test of the importance of connexin quality vs. quantity in intercellular communication (74, 106). The observed functional abnormalities have demonstrated the stringency of connexin-channel requirements in different organs. Finally, the impact of connexins on organ homeostasis has been conclusively illustrated by the restricted phenotypes associated to gene deletions in mouse models and by the finding that mutations in connexin proteins underlie a variety of human genetic diseases, including different forms of deafness, demyelinating neuropathies, skin disorders, and lens cataracts (42).

After a period of relative uncertainty about the molecular identity of the protein subunits of invertebrate gap-junction channels, it has become clear that they are formed by innexins (which stands for invertebrate analogs of the connexins), a family of proteins that share no primary sequence homology but only structural similarity with connexins (5, 72). In total, 25 Caenorhabditis elegans (Ce-INX) and 8 Drosophila melanogaster (Dm-Inx) innexins have been identified, thereby paving the way for the application of genetic tools in the study of their function. Mutant analysis and molecular characterization have shown that innexins are not functionally equivalent and are engaged in similar roles to connexins, for example in synaptic transmission, embryonic and postembryonic development, and morphogenesis (4, 28, 35, 58, 90, 91). In keeping with the basic properties of connexin channels, some innexins can form both heteromeric and heterotypic channels, are gated by transjunctional voltage, modulated by intracellular pH, and permeable to the same fluorescent dyes used in vertebrate studies (35, 55, 73). In contrast, no evidence for active hemichannels (or innexons) has been obtained so far.

**FIGURE 1.** Comparative analysis of the membrane topology of innexins, pannexins, and connexins, the three families of gap-junction proteins. The schematic drawing depicts the predicted arrangement relative to the plasma membrane of one representative member of each protein family: C. elegans innexin-3 (INX-3; top), rat pannexin1 (Panx1; middle), and rat connexin43 (Cx43; bottom). All are four-pass transmembrane proteins, with intracellular amino- (NH<sub>2</sub>) and carboxy- (COOH) termini and similar molecular mass (in kDa: 48.9, 48.1, and 43 for INX-3, Panx1, and Cx43, respectively). The extracellular domains contain regularly spaced cysteine residues (2 for innexins and pannexins, 3 for connexins; yellow dots), whose positions are conserved within but not between families. Differences in the length of the various domains, as deduced from cDNA sequence analysis of INX-3, Panx1, and Cx43, are shown but not drawn to exact scale.
The molecular characterization of the innexin family, although providing an explanation to the failed attempts to clone connexins in the best studied invertebrate animal models, spurred the idea that innexin-like genes could be present in vertebrates. Using PCR with degenerate primers, Panchin and coworkers (69) cloned a molluscan innexin and used this new sequence for a BLAST search of available databases. Mining of the human genome revealed the presence of two putative innexin-like sequences, thus raising the possibility that these genes, collectively called pannexins, may be candidate gap-junction proteins (69). There are currently three pannexins in human and rodent genomes, and orthologous sequences have been identified in zebrafish and Ciona intestinalis, an invertebrate chordate (3, 14, 81). Pannexins are referred to with the abbreviation "Panx," followed by progressive Arabic numbers that distinguish them. Despite the lack of significant sequence homology (see below), strong similarities exist at the structural and functional level with canonical gap-junction proteins. Thus pannexins share the same membrane topology with innexins and connexins as well as the hallmark of regularly spaced cysteine residues in the two extracellular loops connecting the transmembrane domains (FIGURE 1). Whereas the connexins contain three such residues, pannexins contain only two, thus resembling in this respect innexins, although the spacing of the cysteine residues in the second extracellular loop of pannexins diverges from that of innexins (52). Pannexins have since left the role of candidate gap-junction genes, since their ability to assemble active hemichannels and intercellular channels (in both homomeric and heteromeric configurations) has been subsequently verified by functional expression in Xenopus oocytes (2, 14).

The addition of a new group of genes has stirred some debate on their evolutionary relationship to the more established families of gap-junction proteins and, consequently, has raised the issue of nomenclature. On the basis of a tenuous degree of similarity to selected innexin protein domains, Panchin et al. (69) postulated that these new genes belonged to the innexin family and suggested to rename all of them "pannexins" (from the Greek "pan," neuter of the adjective "pas," which means "all," "whole," "entire") to reflect their broad expression in both protostomes and deuterostomes. However, multiple alignments of pannexins with a representative group of innexins and connexins over their entire sequence (removing only the more divergent portions of the middle cytoplasmic loop and COOH-terminal tail) failed to reveal a significant level of homology that would justify inclusion into either one family of proteins (14, 107). In fact, pannexins can be clearly set apart, since they do not share either the innexin signature motif or
Functional properties of pannexin channels

The demonstration that pannexins are channel-forming proteins endowed with several connexin-like properties has been obtained using the *Xenopus* oocyte expression system (2, 13, 14). The evidence can be summarized as follows. First, injection of synthetic RNA for rat Panx1 results in the development of nonspecific, voltage-activated currents in the nonjunctional plasma membrane, indicating that this pannexin can assemble homomeric hemichannels. Second, Panx1 hemichannels are permeable to small molecules. Third, Panx1 shows the ability to form function-

---

### Table 1. Predicted pannexin phosphorylation sites

<table>
<thead>
<tr>
<th>Amino Acid Position (Domain)</th>
<th>Human (Score)</th>
<th>Mouse (Score)</th>
<th>Rat (Score)</th>
<th>Type of Kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Panx1, serine phosphorylation consensus sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>343/344 (C-ter)</td>
<td>SEVKSYKCL (0.996)</td>
<td>SELKSYSCL (0.996)</td>
<td>SELKSYSYCL (0.996)</td>
<td>PKC</td>
</tr>
<tr>
<td><strong>Panx2, serine phosphorylation consensus sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>142 (ML)</td>
<td>TRTLSLNF (0.928)</td>
<td>TRTLSLNF (0.928)</td>
<td>TRTLSLNF (0.928)</td>
<td>RSK, PKA</td>
</tr>
<tr>
<td>329/330 (C-ter)</td>
<td>QWRRSQFCD (0.986)</td>
<td>QWRRSQFCD (0.986)</td>
<td>QWRRSQFCD (0.986)</td>
<td>DNAPK, ATM, PKA</td>
</tr>
<tr>
<td>379/380 (C-ter)</td>
<td>ALAASNHA (0.901)</td>
<td>ALAASNHA (0.965)</td>
<td>ALAASNHA (0.965)</td>
<td>cdc2 (human)</td>
</tr>
<tr>
<td>390/391 (C-ter)</td>
<td>TVRSQGVT (0.995)</td>
<td>TVRSQGVT (0.996)</td>
<td>TVRSQGVT (0.996)</td>
<td>PKA</td>
</tr>
<tr>
<td>445/446 (C-ter)</td>
<td>RENSKAEK (0.915)</td>
<td>RENSKAEK (0.981)</td>
<td>RENSKAEK (0.981)</td>
<td>CKII, PKC, CKK</td>
</tr>
<tr>
<td>531 (C-ter)</td>
<td>PARSQEGG (0.952)</td>
<td>PARSQEGG (0.952)</td>
<td>PARSQEGG (0.952)</td>
<td>DNAPK, ATM</td>
</tr>
<tr>
<td><strong>Panx2, threonine phosphorylation consensus sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>178 (ML)</td>
<td>GPGITEREK (0.969)</td>
<td>GPGITEREK (0.969)</td>
<td>GPGITEREK (0.969)</td>
<td>CKII</td>
</tr>
<tr>
<td>386/387 (C-ter)</td>
<td>DATPTVRS (0.966)</td>
<td>DATPTVRS (0.955)</td>
<td>DATPTVRS (0.955)</td>
<td>PKC</td>
</tr>
<tr>
<td>394/395 (C-ter)</td>
<td>SGVTQVDVS (0.944)</td>
<td>SGVTQVDVS (0.942)</td>
<td>SGVTQVDVS (0.942)</td>
<td>CKII (all), PKC (mouse, rat)</td>
</tr>
<tr>
<td>457/458 (C-ter)</td>
<td>ARRKATATD (0.975)</td>
<td>ARRKATATD (0.985)</td>
<td>ARRKATATD (0.985)</td>
<td>RSK, PKA, PKG</td>
</tr>
<tr>
<td><strong>Panx3, serine phosphorylation consensus sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 (ML)</td>
<td>ELDKSYNRS (0.960)</td>
<td>ELDKSYNRS (0.960)</td>
<td>ELDKSYNRS (0.960)</td>
<td>PKA, PKC, PKG, cdc2</td>
</tr>
<tr>
<td>168 (ML)</td>
<td>IRQSSDPY (0.984)</td>
<td>IRQSSDPY (0.935)</td>
<td>IRQSSDPY (0.992)</td>
<td>PKG</td>
</tr>
<tr>
<td>303 (C-ter)</td>
<td>KRLSYYEM (0.996)</td>
<td>KRLSYYEM (0.965)</td>
<td>KRLSYYEM (0.995)</td>
<td>RSK (all), CKII (mouse)</td>
</tr>
<tr>
<td><strong>Panx3, tyrosine phosphorylation consensus sites</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>186 (ML)</td>
<td>RKERYFEPF (0.946)</td>
<td>RKERYFEPF (0.946)</td>
<td>RKERYFEPF (0.946)</td>
<td>EGF receptor</td>
</tr>
</tbody>
</table>

Pannexin sequences in FASTA format were pasted into NetPhos 2.0 server (http://www.cbs.dtu.dk/services/NetPhos/) and analyzed for the presence of consensus sites for serine, threonine, and tyrosine phosphorylation. The output score is a value in the range between 0.000 and 1.000 (minimal to maximal). Only phosphorylation sites predicted with a score greater than 0.9 and conserved in all three sequences (human, mouse, and rat) are shown. Kinase specificity was analyzed in NetPhosK 1.0 server (http://www.cbs.dtu.dk/services/NetPhosK). ML, middle intracellular loop; C-ter, COOH-terminal.
ally competent intercellular channels. Finally, pannexin hemichannels are closed by commonly used gap-junctional blockers. These findings are discussed below.

The observation of active pannexin hemichannels is relevant, because there is mounting evidence that connexins might also function as unpaired connexons in a variety of different physiological and pathological processes, including metabolic inhibition, apoptosis, bacterial pathogenesis and the response to mechanical stress, and, more recently, the activation of microglia after local brain injury (29, 36, 47). In addition, it is commonly accepted that data obtained from hemichannel experiments (e.g., ionic selectivity, permeability, and voltage-gating behavior) can be extrapolated to predict the properties of intercellular channels (17, 88). Panx1 hemichannels have a large unitary conductance of ~500 pS, and at least four substrates (with 5%, 25%, 30%, and 90% of the maximal conductance) have been observed in single channel recordings (2). In contrast to Panx1, neither Panx2 nor Panx3 form functional homomeric channels (FIGURE 2).

It is well known that certain connexin isoforms, often when expressed in the same tissue, are able to form heteromeric channels with different physiological properties from those featured in homomeric configuration (9, 27, 54). There is both biochemical (co-immunoprecipitation) and electrophysiological evidence to propose that Panx1 interacts with Panx2 (but not Panx3) into heteromeric Panx1/Panx2 channels, which are characterized by a reduced current amplitude and modified voltage-gating kinetics with respect to homomeric Panx1 hemichannels (14). Some differences between pannexins and connexins, however, have already emerged. For example, a distinct feature reported for putative connexin hemichannels is that they are gated by divalent cations, such as Ca^{2+} or Mg^{2+}, so that lowering of extracellular Ca^{2+} concentration increases the open-state probability, whereas at higher physiological Ca^{2+} levels the frequency of channel opening is low (32, 37, 46). By contrast, the opening of pannexin hemichannels is totally insensitive to external Ca^{2+} over a wide range of concentrations (13).

Connexin channels are permeable to second messengers involved in cell signaling (such as cAMP, Ca^{2+}, and inositol trisphosphate), neurotransmitters, some nucleotides, and other specific metabolites (26, 75, 80, 93, 99, 110). More recently, it has been demonstrated that the connexin isoform can influence gap-junctional permeability to such natural metabolites (9, 44, 65). Bao and coworkers (2) have tested whether Panx1 hemichannels are also permeable to molecules other than current-carrying ions and have shown that they allow the passage of ATP. A much debated idea is the contribution of hemichannels to Ca^{2+} waves (7, 47), a form of intercellular signaling observed in different tissues, including brain, which consists of the coordinated propagation of Ca^{2+} transients across a population of cells (see below). Since the release of ATP in the extracellular space through hemichannels is an important component of Ca^{2+} waves, pannexins are additional candidate proteins whose role in this phenomenon should be tested in the future. Interestingly, Panx1 can be opened by mechanical stress and, in contrast to the properties of most putative connexin hemichannels, is active at physiological extracellular Ca^{2+} concentrations. Thus pannexin hemichannels fulfill several criteria that are expected for the molecular substrates of this form of cell-cell communication.

The ability to form intercellular channels has been tested in paired oocytes, where two cells are manually brought in contact after the injection of specific RNA. These experiments have demonstrated that Panx1 alone and in combination with Panx2 induces the assembly of intercellular channels, whereas Panx2 and Panx3 alone fail to do so (14). Both Panx1 and Panx1/Panx2 pairs display a remarkable insensitivity to transjunctional potentials of opposite polarities (FIGURE 2). Thus, with a driving force ≈ ±60 mV, junctional currents vary linearly with voltage, whereas, at higher transjunctional potentials, conductance of pannexin intercellular channels display only a very modest reduction of the initial values. However, this behavior is not unusual, and a whole spectrum of voltage-gating properties has been reported for other connexin-based gap-junction channels (49). Despite their apparent similarity, the possibility that pannexins exist as homomeric as well as heteromeric channels suggests that pannexin channels with distinct molecular composition may underlie different tasks.

Information on the mechanisms that regulate the activity of pannexin channels is still in its infancy. It is safe to speculate that gating is likely to be modulated by posttranslational modifications, chiefly phosphorylation, as is the case for most connexins that have been analyzed (56, 61–63). Indeed, both the middle cytoplasmic loop and carboxyl-terminal tail present several consensus sites for distinct protein kinases that have been conserved in rodent and human sequences (Table 1). Knowledge of the pharmacological sensitivity of pannexin channels is also crucial to understand their role in normal tissue function. Pannexin hemichannels display a remarkable sensitivity to compounds derived from the licorice root (e.g., carbenoxolone and glycyrrhetinic acid) (13), which have been used over the past decade to block connexin hemichannels and gap junctions. In contrast, the aryloxybenzoate flufenamic acid, a member of a large family of chloride-channel blockers that has recently been shown to close connexins channels (89), exerted only a modest inhibitory effect. The opposite is true in the case of Cx46, thus indicating that gap-junction blockers are able to selectively modulate pannexin and connexin channels. In addition, the mechanism of action of these two drugs on pannexins appears to be different, with carbenoxolone inhibiting both current
amplitude and kinetics of channel closure, whereas flufenamic acid only decreases peak current values (13). Further studies are needed to characterize the pharmacology of pannexins and to assess whether specific molecules may discriminate them from connexin channels.

**Expression Pattern of Pannexins**

Northern blot analysis indicates that Panx1 and Panx2 transcripts are detected in many rodent tissues, including brain and spinal cord (where Panx2 is particularly abundant), eye, thyroid, prostate, and kidney (14, 77, 102, 104). The widespread distribution of Panx1 has been confirmed by probing human tissues, with the highest levels being found in heart, gonads, and skeletal muscle (3). These results are at variance with those reported in rodents, in that no signal was detected in skeletal muscle and heart by Northern blot (14), whereas only a weak band was amplified by RT-PCR from cardiac mRNA (77). The presence of Panx1 transcripts in adult human skeletal muscle is puzzling given the syncytial nature of this tissue, which does not form gap junctions. If Panx1 protein is indeed expressed, it may function as a hemichannel or subserve a different role. In addition, Panx2 distribution in man appears restricted to brain (3), whereas it is co-expressed with Panx1 in many organs in rodents (14). Further studies will help resolve whether these few discrepancies reflect true species differences.

Panx3 presents the most restricted pattern of distribution and has been detected only in skin, which is devoid of Panx1 and Panx2 mRNA (14). Although we could never detect a positive signal with Panx3 probes in the brain (whether by Northern blots or in situ hybridization), it should be pointed out that a positive cDNA clone was isolated from a *postnatal day 15* rat hippocampal library (14). This may imply that extremely low levels of transcripts are present, an interpretation consistent with the weak positive signal detected in RT-PCR experiments performed on mRNA isolated from human hippocampus.

The expression pattern of both Panx1 and Panx2 has provided the impetus for more focused investigations in the brain, primarily because of a regain of interest in deciphering the role played by gap junctions, which are the morphological correlate of electrical synapses. It is now believed that this form of synaptic communication has a major role in the formation of many neuronal circuits during development as well as in synchronizing large neuronal ensembles at different frequency bands (8, 24, 50, 85) that have been proposed to underlie a variety of cognitive processes, such as perception, memory, and learning (83). In the central nervous system, connexins are found in many regions (6, 85), although compelling functional and morphological data have been collected for only a few areas, such as retina, hippocampus, inferior olivary nucleus, olfactory bulb, thalamic reticular nuclei, and neocortex (11, 22, 31, 40, 41, 43, 76, 84, 98, 100). Thus a detailed knowledge of the profile of pannexin expression in the brain (whether in neurons or glia, or in interneurons or pyramidal cells) will be instrumental to provide a rational basis for future functional studies in vivo or in brain slices.

By using a combination of quantitative real-time PCR, in situ hybridization, and immunocytochemistry, a first comprehensive atlas of the spatio-temporal distribution of Panx1 and Panx2 in the central nervous system has been obtained (77, 102). Both genes have a widespread and similar distribution (FIGURE 3), but their relative abundance is inversely regulated during development. Panx1 expression peaks at around embryonic day 18 to postnatal day 1 and then declines considerably in adult animals. In contrast, Panx 2 is present at very low levels at all embryonic stages and then increases dramatically during postnatal development, peaking at postnatal day 15 (77, 102).

The predominant neuronal nature of pannexin...
expression has been confirmed by showing that all Panx1- and Panx2-positive cells are labeled with NeuN, a mouse monoclonal antibody that recognizes almost all neuronal populations, whereas they are not stained by antibodies recognizing glial fibrillary acidic protein, which is an astrocyte-specific intermediate filament (77, 102). Panx1 and Panx2 transcripts are particularly abundant in cortex (especially prefrontal and entorhinal areas), hippocampus, reticular thalamus, motor neurons of brain stem and midbrain, the supraoptic and suprachiasmatic nuclei of the hypothalamus, and cerebellum (with an intense labeling of Purkinje cells and also of some somata in the granule layer, presumably Golgi cells) (14, 77, 102, 104). Intriguingly, pannexins have also been detected in areas with previously unrecognized gap junctions, raising the possibility either that they fulfill a function independent of intercellular coupling or that electrical synapses are more widespread and dynamically regulated than previously appreciated.

In the hippocampal formation (Cornus Ammoni and the dentate gyrus), Panx1 and Panx2 strongly label the majority, if not all, of principal cells in the pyramidal layer. Furthermore, in the strata oriens, lacunosum moleculare, and radiatum, there are scattered pannexin-positive cells that, based on their location, can be inferred to be GABAergic interneurons (14, 77, 102). Thus pannexins are present in both excitatory and inhibitory as well as projection and local circuit neurons in the cortex and hippocampus, whereas Cx36 expression, the main neuronal connexin, appears restricted to inhibitory GABAergic interneurons in the adult (6, 51).

GABAergic interneurons comprise a heterogeneous population that can be subdivided into many cell types according to anatomical and neurochemical criteria, with the presence of specific calcium-binding proteins being one of them (39). To define the neuronal subtypes expressing pannexins, in situ hybridization has been combined with immunostaining. The vast majority of parvalbumin-positive cells co-express Panx1 and Panx2 in olfactory bulb, cortex, hippocampus, and cerebellum, whereas the percentage of co-localization with calretinin and calbindin appears variable, depending on the area under investigation (77, 102). Thus both Panx1 and Panx2 transcripts are expressed in specific subtypes of interneurons that are now recognized to play a crucial role in regulating the complex interactions with principal cells, including the synchronization of cortical network activity (see below). The expression of Panx2 and its interaction with Panx1 could be an elegant mechanism to modulate pannexin channels, since heteromeric assemblies result in lower macroscopic currents compared with homomeric channels.

Some differences in the cellular distribution of Panx1 and Panx2 mRNA have emerged. For example, we have found that Panx1 expressing cells are present in cerebellar white matter where Panx2 expression is absent (Ref. 14, but see also Ref. 77). The labeling of Panx1 in white matter is not restricted to the cerebellum but is also observed in other structures (e.g., corpus callosum, limbria fornix) that, similarly, are also devoid of Panx2 expression (14). The cellular identity of these signals remains to be determined. Taken together, these observations suggest that the role of Panx1 and Panx2 may vary with the maturation of the neuronal circuitry.

Which Roles for Pannexin Channels?

The presence of two unrelated protein families that underlie similar functions is intriguing, because it raises the possibility that cell-specific regulatory properties and gating mechanisms are achieved through this multiplicity. Although we still lack the crucial proof that pannexins provide a distinct form of intercellular communication via hemi- or intercellular channels, we would like to speculate on their possible role in the nervous system by integrating the available data into testable working hypotheses.

Pannexins and synchronous activity in the brain

The functional implication of gap junctions between coupled neurons has primarily been examined in the context of the speed of signal transmission and of the precise temporal synchronization provided by electrical synapses (24, 50, 57). Brain oscillations occur at different frequency bands and reflect the periodic variation of the membrane potential of neuronal networks (20, 95). Rhythmic electrical activity is believed to be the basis of the “binding theory,” which posits that synchronization of cell firing brings into a common representation the elementary features of an object that are distributed in different brain areas (83). Interestingly, different frequencies have been linked to different behavioral states. For example, the acquisition of information represented by alterations in synaptic strength may take place during the theta (9–12 Hz) and gamma (40–90 Hz) rhythms, which are recorded during exploration and sleep, whereas ultrafast oscillations (150–200 Hz), or ripples, that occur in the immobile awake and sleeping animal may be associated with the consolidation of these patterns and their transfer to other brain structures (19, 70). This general, two-stage framework of memory trace consolidation is supported by several experiments and computational models (20).

Pharmacologically induced gamma oscillations depend on both chemical synaptic inhibition and gap-junctional coupling (96, 108). Ultrafast oscillations, in contrast, are regarded as an emergent property of a coupled pyramidal cell network. In vitro, they have been shown to occur in the absence of chemical neurotransmission and require axo-axonal coupling.
signals by releasing glutamate and ATP (7). Thus, other than the classical theory of gap junction-dependent Ca\textsuperscript{2+} waves (21, 101), functional hemichannels that mediate the release of ATP into the extracellular space are also regarded as key components of this phenomenon (26, 48, 93). ATP, in turn, binds to metabotropic P2Y receptors, leading to an increase in inositol 1,4,5-trisphosphate, which releases Ca\textsuperscript{2+} from intracellular stores. By an as yet poorly defined mechanism, this Ca\textsuperscript{2+} elevation activates hemichannels (12), leading to the release of ATP, which propagates a regenerative signal to neighboring cells in the form of a wave.

Several recent findings make a case for the involvement of hemichannels in the regulation of neuronal proliferation in the developing brain. Weissman and coworkers (105) have identified Ca\textsuperscript{2+} waves that propagate through radial glial cells in the ventricular zone at embryonic day 16. Radial glial cells have long been known to play a structural role in guiding neuronal migration (111), and, more recently, they have been shown to be an important reservoir of neuronal progenitor cells. In this work, Ca\textsuperscript{2+} waves were shown to occur spontaneously and propagate in keeping with the hemichannel theory. Thus they are initiated by the opening of hemichannels that mediate the release of ATP, which, in turn, activates metabotropic P2Y receptors to spread the signal. Furthermore, the application of the connexin blockers carbenoxolone and flufenamic acid could abolish the spontaneous wave propagation effectively (105). Similarly, ATP released from the pigment epithelium through hemichannels (supplement 110) interacts with metabotropic purinergic receptors...

**Panx1 hemichannels (red) are permeable to the nucleotide ATP.**

**ATP interacts with metabotropic purinergic receptors...**

**Either Ca\textsuperscript{2+} or IP\textsubscript{3} can open Panx1 to release ATP and transmission of a Ca\textsuperscript{2+} wave.**

**IP\textsubscript{3} induces release of Ca\textsuperscript{2+} from ER.**

**Carbenoxolone and flufenamic acid inhibit Panx1.**

**FIGURE 4. Hypothetical role of pannexin hemichannels in the propagation of calcium (Ca\textsuperscript{2+}) signals**

Panx1 hemichannels (red) are permeable to the nucleotide ATP and should be considered as good candidates to mediate the transfer of Ca\textsuperscript{2+} signals to neighboring cells, thereby participating in a number of processes during development and in the adult (see Pannexins and Ca\textsuperscript{2+} waves). The released ATP would interact with metabotropic purinergic receptors (P2Y, blue), bringing about the production of the second messenger inositol 1,4,5-trisphosphate (IP\textsubscript{3}; green circles), which induces Ca\textsuperscript{2+} release from the endoplasmic reticulum (ER). Either IP\textsubscript{3} or a Ca\textsuperscript{2+}-dependent process would then trigger the opening of Panx1 hemichannels, ensuring the release of ATP and the transmission of a Ca\textsuperscript{2+} wave.
posedly made of Cx43) induces Ca\(^{2+}\) transients in neural retinal progenitor cells that increase the rate of proliferation during retinal development. Of note, these hemichannels appear to be ideally positioned in the plasma membranes of the pigment epithelium facing the neural retina (71).

Although most publications have thus far suspected Cx43 as the main player of hemichannel-mediated Ca\(^{2+}\) waves in the brain (7, 26), several findings indicate that Cx43 hemichannel activation can only be observed by combining low extracellular Ca\(^{2+}\) concentrations with large membrane depolarizations or during stressful conditions (25, 59, 93). Furthermore, in spinal cord astrocytes, the velocity of intercellular wave propagation is not altered in Cx43 knockout mice (82). The molecular identity of hemichannels remains controversial, and it is reasonable, therefore, to postulate that other proteins should be carefully considered as an alternative, or in addition, to Cx43. We would like to speculate, based on the developmental profile (high expression in the embryonic brain and in the retina) and biophysical properties [activation at physiological Ca\(^{2+}\) concentrations, sensitivity to gap-junction blockers, permeability to ATP and Ca\(^{2+}\)-induced opening (2, 13)], that Panx1 hemichannels may be involved in the initiation and propagation of Ca\(^{2+}\) waves (FIGURE 4). Furthermore, it has been reported that an increase in cytoplasmic-free Ca\(^{2+}\) concentrations results in the opening of Panx1 hemichannels, which is accompanied by ATP release (60).

**Pannexins and disease**

Molecular genetics has revolutionized our appreciation of gap junctions by showing that mutations in connexin genes are linked to various hereditary disorders in humans (42). Moreover, several studies have found correlative evidence to implicate gap junctions and hemichannels in the pathology of brain diseases (64). Although it may seem premature to speculate on the involvement of pannexins in disease, we think that the proposed function of pannexin-based channels in the brain could be discussed in the context of two types of pathologies: epilepsy and schizophrenia.

Conventional electroencephalograms obtained from human subjects cover a range of frequencies composed of between 0.5 and 30 Hz and, therefore, cut off the high-frequency activity. Traub and coworkers (97) have documented the existence of high-frequency oscillatory phenomena before the onset and during seizures with the help of subdural EEG recordings from human epileptic neocortex. Similarly, very fast oscillations were observed before, between, and after epileptiform bursts in rat hippocampal slices. These seizure-like events were recorded in the absence of chemical synaptic activity and could be suppressed with carbamazepine, indicating that gap junctions are involved. Of note, in silico simulations of high-frequency oscillations were obtained only when axo-axonal gap junctions between pyramidal cells were included (97). Thus the possible involvement of pannexins in epileptogenesis should be considered in future work.

Schizophrenic disorders are characterized by a wide range of symptoms that reflect alterations in cognitive, psychomotor, and emotional processes. One theory linking electrical synapses to schizophrenia is based on the proposal that the function of the nucleus accumbens may be disturbed in schizophrenia (66). Because the nucleus accumbens is a site where a large number of inputs converge, the idea that gap junctions may be required for its synchronous activity has obviously surfaced. Although electron microscopy images have not revealed typical gap-junction structures within the nucleus accumbens, close membrane appositions similar to those observed in gap junctions have been found (33). The reported distribution of Panx1 is consistent with a sparse distribution of pannexin channels in this nucleus (77), where they could modulate the activity of neuronal ensembles. Current views of schizophrenia emphasize the dysfunction of neural microcircuits as the underlying basis of this disorder. A causal relationship between schizophrenic symptoms and disruption of neural synchrony has been suggested by a recent paper that reported a lower frequency of gamma-band oscillations in schizophrenic patients that were confronted with visual Gestalt stimuli (87). Thus it is worth examining all neuronal gap-junction proteins as candidate genes in the familiar forms of schizophrenia.

**Conclusions**

The unexpected discovery of an additional family of putative gap-junction proteins has brought a new set of players on the stage of cell-to-cell communication. Evolutionary considerations aside, the initial characterization of pannexins at the functional level supports their involvement in signaling via hemi- and/or intercellular channels. It should be stressed, however, that a number of crucial questions remain to be answered. First and most importantly, it must be demonstrated that pannexins form channels in vivo and that specific functional deficits result by repressing their activity through genetic and pharmacological approaches. This may prove quite difficult if one considers that only few axo-axonal gap junction channels may suffice to sustain high-frequency rhythms in the hippocampus. Other pressing issues include a detailed understanding of the oligomeric structure of pannexin channels (are they hexamers as connexins?), the visualization of their subcellular distribution (do they form bona fide gap junctions?), and the identification of candidate interacting proteins (18). A panoply of tools is being developed to precisely define the unique contributions of pannexins to cellular functions.

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References


