Abstract

Cell-to-cell communication through gap junctions exists in most animal cells and is essential for many important biological processes including rapid transmission of electric signals to coordinate contraction of cardiac and smooth muscle, the intercellular propagation of \( \text{Ca}^{2+} \) waves and synchronization of physiological processes between adjacent cells within a tissue. Recent studies have shown that connexins (Cx) can have either direct or indirect interactions with other plasma membrane ion channels or membrane transport proteins with important functional consequences. For example, in tissues most severely affected by cystic fibrosis (CF), activation of the CF Transmembrane Conductance Regulator (CFTR) has been shown to influence connexin function. Moreover, a direct interaction between Cx45.6 and the Major Intrinsic Protein/AQP0 in lens appears to influence the process of cell differentiation whereas interactions between aquaporin 4 (AQP4) and Cx43 in mouse astrocytes may coordinate the intercellular movement of ions and water between astrocytes. In this review, we discuss evidence supporting interactions between Cx and membrane channels/transporters including CFTR, aquaporins, ionotropic glutamate receptors, and between pannexin1, another class of putative gap-junction-forming proteins, and \( \text{Kv}_{\beta_3} \), a regulatory \( \beta \)-subunit of voltage gated potassium channels. Although the precise molecular nature of these interactions has yet to be defined, their consequences may be critical for normal tissue homeostasis.

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Keywords: CFTR; Aquaporin; Glutamate receptors; Pannexin

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1. Introduction

Gap junction intercellular communication (GJIC), involving direct exchange of information between adjacent cells, was first described 40 years ago (Furshpan and Potter, 1959; Dewey and Barr, 1962; Kumar and Gilula, 1996; Goodenough et al., 1996; Spray, 1996; Harris, 2001; Evans and Martin, 2002; Willecke et al., 2002). The structure responsible for this direct exchange, which was termed “gap junction” by Revel, 1968, offers a third pathway for intercellular communication with the synapse and the neuromuscular junction (Wells and Bonetta, 2005). Gap junctions consist of aggregates of transmembrane hemichannels (or connexons), that dock to similar connexons on the neighboring cell. Three-dimensional structure of a recombinant gap junction channel revealed that opposing connexons are staggered by 30° and packed in the intercellular gap (Perkins et al., 1998; Unger et al., 1999), resulting in a tight seal between the two hemichannels. The wall of each connexon is formed of six protein subunits, termed connexins (Cx), creating a 2 nm-diameter aqueous pore that allows diffusion of molecules of about 1000 Da between the cytoplasm of adjacent cells (Kumar and Gilula, 1996; Goodenough et al., 1996; Spray, 1996; Harris, 2001; Evans and Martin, 2002; Willecke et al., 2002). The commonly used nomenclature distinguishes Cx by their molecular mass deduced from their respective cDNAs.

Gap junctions drive numerous important biological processes including rapid transmission of electric signals to coordinate contraction of cardiac muscle and smooth muscle, the intercellular propagation of Ca²⁺ waves and synchronization of cell activities within a cell cluster. In addition, relationships between transport mechanisms that contribute to electrical properties of interconnected cells and the function of gap junctions has been widely reported. For example, GJIC is reduced by blockers of the Na⁺/K⁺-ATPase and inhibition of GJIC is associated with membrane depolarization and/or altered electrical activity of interconnected cells (Ledbetter and Gatto, 2003; Hervé and Sarhouille, 2005). Another example involves the recently discovered role of Cx36-mediated intercellular communication in the regulation of electrical activity, intracellular Ca²⁺ changes and insulin secretion (Göpel et al., 1999; Ravier et al., 2005). Thus, these findings suggest that Cx and other membrane channels or transporters act in concert to regulate tissue homeostasis. The nature of these interactions may be direct or indirect depending on the specific transport proteins involved. In this review, we summarize the evidence obtained so far on interactions between Cx and membrane channels/transporters and discuss possible functional implications as well as what is known about the molecular basis of these interactions.

2. Relationship between CFTR functions and GJIC

2.1. Regulation of Cx45 activity by cAMP in CFTR and ΔF508 CFTR expressing cells

Cystic fibrosis (CF) is a condition caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. The gene product, a cAMP and ATP regulated Cl⁻ channel, is mostly expressed in the apical plasma membrane of secretory and reabsorptive epithelia of affected organs, allowing the transepithelial movement of water and solutes. Other observations indicate a more widespread CFTR gene expression than in epithelial cells and suggest that this channel protein may function in most human cells to help maintain...
cellular homeostasis (Yoshimura et al., 1991; Abraham et al., 2001; Assef et al., 2003, 2005). The first suggestion for a role of GJIC in CF came from experiments aimed at correcting the CF defect by insertion of wild-type CFTR gene (Johnson et al., 1992; Zabner et al., 1994). These experiments showed that by correcting as few as 6–20% of CF cells, it was possible to restore their normal fluid transport properties (Johnson et al., 1992). It has been proposed that ionic and/or metabolic coupling through gap junction channels may serve for amplification of the functional effects of the corrected cells. Previous studies have reported that molecules which activate (cAMP) or inhibit (ATP depletion) CFTR channels modulate gap junction connectivity (Chanson et al., 1996; Brezillon et al., 1997). Conversely, disruption of GJIC has been associated with altered functions in tissues in which manifestations of CF occur (Chanson and Suter, 2001). The question of whether epithelial cells devoid of functional CFTR exhibit abnormal GJIC has been addressed recently.

The preferred sites of CFTR expression in the pancreas are duct cells where CFTR functions as a Cl\(^{-}\)/\(C0\) channel and a regulator of the \(HCO3\^-/C0\)/\(Cl\^-/C0\) exchanger (Choi et al., 2001; O’Reilly et al., 2002). In CF, the first event in chronic pancreatitis may be the dysfunction of duct cells, leading to decreased bicarbonate secretion, and thereby luminal acidification, resulting in secondary injuries of exocrine acinar cells (Kopelman et al., 1988; Freedman, 1998). The comparison of CFTR and gap junction channel activity was studied between a pancreatic duct cell line (CFPAC-1) derived from a patient with CF and its counterpart stably expressing wild-type CFTR (PLJ-CFTR). In this study, agents elevating intracellular cAMP concentration or specifically activating PKA opened within minutes CFTR and gap junction channels. In contrast, these agents had no effect on GJIC or Cl\(^{-}\) transport in CF cells (Chanson et al., 1999). The effect of cAMP on GJIC, which was caused by an increase in single-channel activity but not in unitary conductance of Cx45 gap junction channels, was not prevented by exposing CFTR-expressing cells to a Cl\(^{-}\) channel blocker. These observations suggested an interaction between CFTR and Cx.

### 2.2. Effects of CFTR expression on Cx45 voltage-sensitivity and gating

The interaction between CFTR and gap junction channels was since confirmed by co-expression of the two channels in paired Xenopus oocytes (Kotsias and Peracchia, 2005). With co-expression of CFTR and Cx45, the application of forskolin to paired oocytes, clamped at −20 mV and subjected to −40 mV transjunctional voltage (\(V_j\)) pulses of 12 s duration, induces a typical outward current (\(I_m\)) and increases junctional conductance (\(G_j\)) by 60–100%. In addition, the presence of CFTR reduces the \(V_j\) sensitivity of Cx45 channels without affecting the kinetics of junctional current inactivation. The drop in voltage sensitivity is further enhanced by forskolin application, as shown by a sizable increase in the ratio between steady-state and peak junctional conductance (\(G_{jSS}/G_{jPK}\)) at the pulse. These data indicate that CFTR influences both conductance and voltage sensitivity of Cx45 channels. The increase in peak conductance is likely to reflect a reversible increase in open channel probability, as shown in small cells studied by dual whole-cell patch clamp (Chanson et al., 1999).

Gap junction channels are known to be gated by voltage and increased [\(Ca^{2+}\)]\(_i\) or [\(H^+\)]\(_i\), via molecular mechanisms still largely unclear (Harris, 2001; Peracchia, 2004). At least two voltage sensitive gates have been identified: fast and slow. The fast gate and chemical gate are believed to be distinct, whereas slow gate and chemical gate are likely to be the same (Bukauskas and Peracchia, 1997; Peracchia et al., 1999, 2000). Slow and fast \(V_j\) gates are in series and each hemichannel appears to have both gates. In contrast to the behavior of most connexin channels, the gate activated by voltage in Cx45 channels appears to be preferentially the slow gate (Elenes et al., 2001; Bukauskas et al., 2002). Therefore, it is reasonable to believe that CFTR activation somehow influences the behavior of the slow/chemical gate of Cx45 channels. Significantly, the drop in voltage sensitivity is not accompanied by a change in time constant of single exponential decay of junctional current. This may indicate that CFTR influences the slow gating mechanism of some of the Cx45 channels, rendering them insensitive to voltage. Perhaps, this creates two populations of Cx45 channels: voltage-sensitive and voltage-insensitive.

Interestingly, recent data indicate that CFTR has a similar effect on Cx32 and Cx40 channels and, to a lesser extent, on Cx50 channels (Kotsias et al., 2005). In Cx45, Cx40, Cx32 and Cx50, \(G_{jPK}\) increases by approximately 750%, 560%, 25% and 8%, respectively, and the ratio \(G_{jSS}/G_{jPK}\) by approximately 75%,
43%, 25% and 20%, respectively. This indicates that CFTR may influence the function of gap junctions in a variety of cells in addition to those that express Cx45.

2.3. Regulation of Cx43 activity by TNF-α in CFTR and ΔF508 CFTR expressing cells

Gap junction channel expression and/or connectivity is altered in a number of inflammatory conditions in vitro and in vivo. In vitro, inhibition of GJIC by proinflammatory mediators, such as lipopolysaccharide (LPS), TNF-α, IL-1α or IL-1β has been documented in various primary and immortalized cell types (Sáez et al., 2000; De Maio et al., 2002; Chanson et al., 2005). Interestingly, TNF-α was found to differentially regulate GJIC in airway cell lines of non-CF and CF origin (Chanson et al., 2001). In non-CF airway cells, gap junction channels composed of Cx43 close within 20 min following cytokine exposure. Similar effects on GJIC were measured for LPS and lysophosphatidic acid, a potent ligand for G-coupled membrane receptors. In contrast, these molecules failed to modulate GJIC in the CF airway cell lines (Huang et al., 2003).

The role of CFTR in the regulation of gap junction channel activity during the inflammatory response was examined after correction of the CF phenotype by transfer of wild-type CFTR with replication-defective adenovirus containing the CFTR cDNA (Ad CFTR) (Chanson et al., 2001). Under these conditions, TNF-α-induced down-regulation of GJIC was restored in CF airway cells infected with Ad CFTR but not with adenovirus encoding a reporter gene. Thus, these observations provide additional support to the view that CFTR and Cx interact. In addition, they suggest that expression of CFTR is necessary for the regulation of GJIC by proinflammatory mediators.

2.4. Speculation on mechanisms of Cx43/45 regulation by CFTR

Increasing evidence indicates that CFTR can exist within a multiprotein complex, in which its activity is regulated by interactions with other proteins and/or may confer regulatory properties to signal transduction networks (Guggino, 2004; Li and Naren, 2005). High affinity association of multivalent PDZ domain-containing proteins with the conserved C-terminal TRL motif has been demonstrated. For example, the C-terminal of CFTR forms a binding site for the first PDZ domain of EBP50 (ezrin/radixin/moesin-binding phosphoprotein of 50 kDa), an adapter protein concentrated at the apical membrane of numerous cell types, including airway epithelial cells (Liedtke et al., 2002). EBP50 interacts with ezrin, which acts as an actin binding protein, therefore forming a complex linked to the cytoskeleton. The same EBP50 protein is also involved in the apical targeting of c-Yes (and eventually c-Src), a tyrosine kinase of the Src family, via the YAP65 protein. YAP65 interacts with both EBP50 and c-Yes (Short et al., 1998; Mohler et al., 1999; Moyer et al., 2000).

The capability of CFTR to interact with other intracellular proteins may provide a molecular mechanism for the regulation of gap junction channels. Several indications suggest that the signal transduction pathway involved in the down-regulation of GJIC by TNF-α requires the tyrosine kinase c-Src. First, pharmacological antagonists and expression in non-CF cells of a dominant negative construct of c-Src prevents gap junction channel closure by TNF-α. Second, Cx43 channel closure was also not observed by expressing a connexin mutant lacking tyrosine phosphorylation sites for c-Src (Huang et al., 2003). Thus, these observations indicate that Src links mediators of inflammation to Cx43 gap junction channels and acts as a central element in connecting CFTR with proinflammatory mediators.

What is the molecular basis that underlies the CFTR-c-Src-Cx43 interaction? The data presented above suggest that the activation of c-Src is defective in CF airway cells. Src-family kinases are negatively regulated by the c-Src tyrosine kinase (Csk), which is bound to and activated by Cbp (Csk-binding protein), a broadly expressed transmembrane adapter protein (Kawabuchi et al., 2000). Interestingly, Cbp was found to interact with EBP50, the cytoplasmic adapter that also binds to the last amino acids at the C-terminus of CFTR (Brdickova et al., 2001). Thus, EBP50 and other scaffolding proteins have not only the ability to target CFTR to membrane compartments via ezrin and the actin cytoskeleton, but may also mediate the regulation of CFTR and/or by CFTR of other ion channels via protein–protein or signaling interactions (Moyer et al., 2000; Naren et al., 2003). As suggested in Fig. 1, CFTR may modulate the sensitivity of c-Src to activation by
affecting the Cbp/Csk complex. This is an interesting possibility since it may provide a mechanism for the CFTR-dependent indirect regulation of gap junction channels by cAMP/PKA and c-Src that was observed in pancreatic duct and airway epithelial cell lines (Chanson and Suter, 2001). In addition, such an upstream control of signal transduction pathways may reconcile reports on the pleiotropic effects of CFTR obtained in different cell models (Fig. 2).

3. Functional relationships between Cx and aquaporins

3.1. Connexin and aquaporin-0 interactions in vertebrate lens

The lens is composed of fiber cells which differentiate from lens epithelial cells through a complex program of structural and genetic changes that continues throughout the lifespan of the organism (Al-Ghoul et al., 2003; Graw, 2004). In contrast to other tissues, the lens has no blood supply, thus the fiber cells at the center of the lens are dependent on a gap junction network that facilitates the exchange of electrolytes and metabolites (Al-Ghoul et al., 2003). Developmental studies using chick lens have shown that three connexin proteins (Cx43, Cx45.6 and Cx56) are expressed and form the structural components of gap junctions (Yu and Jiang, 2004). Cx43 is the connexin responsible for GJIC between lens epithelial cells whereas Cx45.6 and Cx56 form the gap junctions that are predominantly associated with fiber cells (Yu and Jiang, 2004). The most abundant membrane protein of lens fiber cells is major intrinsic protein (MIP). MIP does not form gap junction-like channels, but is instead a member of the aquaporin family of membrane proteins designated as aquaporin-0.
Expression of MIP/AQP-0 in *Xenopus* oocytes results in an increase in water permeability which can be regulated by changes in pH and [Ca$^{2+}$] (Chandy et al., 1997; Varadaraj et al., 1999; Nemeth-Cahalan and Hall, 2000; Virkki et al., 2001; Verkman, 2003; Kalman et al., 2006). Interestingly, confocal microscopy studies of the early stages of embryonic chick lens development demonstrated that MIP/AQP-0 consistently co-localized with gap junctions formed by Cx45.6 and Cx56 (Yu and Jiang, 2004). A pull-down analysis of early embryonic chick lens lysates indicated that the C-terminus of MIP/AQP-0 interacts with these two connexin proteins, but co-localization could only be detected in the actively differentiating bow regions of late embryonic and adult lenses. Further molecular analysis revealed that the C-terminus of MIP/AQP-0 specifically interacts with the intracellular loop region of Cx45.6 (Yu and Jiang, 2004; Yu et al., 2005). During the later stages of differentiation this interaction appears to be disrupted because of cleavage of the intracellular loop, resulting in dissociation of the two proteins (Yu et al., 2005). The interaction between MIP/AQP-0 and Cx45.6 does not affect Cx45.6-mediated intercellular communication and thus an understanding of its functional significance will likely require more information about the role of MIP/AQP-0 in fiber cell differentiation.
3.2. Connexin and aquaporin-4 interactions in astrocytes within the brain

Interactions between aquaporin protein family members and Cx are not limited to cells within the lens. Co-regulation of aquaporin-4 (AQP-4) and Cx43 has been reported in primary astrocytes isolated from mouse brain (Rash and Yasumura, 1999; Nicchia et al., 2005). AQP-4 is the major water channel present in the brain, where it has been identified in the perivascular membranes of astrocytes (De Pina-Benabou et al., 2001). Cx43 is the primary gap junction protein expressed in astrocytes and is involved in the clearance of ions/molecules from the active neuropile (Rash et al., 2004). Knockdown (KD) of AQP-4 by RNAi in primary cultures of mouse astrocytes (but not rat or human astrocytes) produces a decrease in Cx43 protein expression (Nicchia et al., 2005). The reduction in Cx43 protein was associated with a significant decrease in functional coupling between the cells consistent with immunocytochemistry results showing reductions in Cx43 labeling at the appositional surfaces of mouse AQP-4 KD astrocytes. Although a direct interaction has not been identified, the positive correlation between the degree of AQP-4 KD and reductions in Cx43 expression and intercellular coupling provides compelling support for a functional relationship between AQP-4 and Cx43. A possible reason for this relationship might be to control intercellular ion and water movements mediated by Cx43 under conditions where AQP-4-dependent water efflux is enhanced or diminished.

4. Functional interactions between Cx35/36 and ionotropic glutamate receptors

Auditory afferent fibers terminating as large myelinated club endings (LMCE) on the lateral dendrite of goldfish Mauthner cells have served as an important model system for the study of electrical transmission between vertebrate neurons (Pereda et al., 2003a). Gap junctions, composed of Cx35, the teleost ortholog of the neuron specific mammalian Cx36, and NMDA receptors are localized at these nerve terminals (Pereda et al, 2003a, 2003b). Although activity-dependent short-term plasticity had been previously characterized at chemical synapses, Pereda and Faber (1996) first demonstrated that electrotonic synapses at LMCEs also exhibit increased conductance in response to sustained cellular activity. Since enhancement of electrotonic conduction requires NMDA receptor activation, it has been proposed that a functional interaction exists between NMDA receptors and Cx35 proteins that form gap junctions at the LMCEs (Pereda et al., 2003a). Moreover, the extensive distribution of NMDA receptors and gap junctions within the mammalian central nervous system (CNS) suggests that this functional interaction might constitute a common property of electrical synapses. Double freeze-fracture replica immunogold labeling using antibodies for Cx35 and the NR-1 subunit of the NMDA receptor in combination with confocal microscopy showed that these proteins exist in close proximity at post synaptic densities (PSDs) localized at the periphery of the LMCEs (Pereda and Faber 1996; Pereda et al., 2003a; Rash et al., 2004). Activity-dependent modulation of electrical coupling appears to require stimulation of the adjacent glutamate receptive regions within the same synapse. Anatomical data suggests that the critical distance for this regulatory interaction is at least equal to the separation between PSDs and the closest gap junction plaque, which is typically within the range of a few nanometers (Pereda et al., 2003a). Freeze fracture studies on various mammalian neurons indicate that distances between PSD and gap junction plaques at the synapse is similar to what has been reported at LMCEs. This observation is consistent with the speculation that gap junction modulation by glutaminergic signaling at adjacent PSDs is possible within the mammalian CNS.

More recently, direct evidence for developmental regulation of electrical synapses by NMDA receptor activation has been obtained in hypothalamic neurons (Okada et al., 2003; Arumugam et al., 2005; Kandler and Thiels, 2005). During synaptic circuit development in the rat medial hypothalamus as well as other brain regions, neuronal communication transitions from electrical coupling via gap junctions to chemical synaptic transmission. Down regulation of the neuronal gap junction protein Cx36 requires NMDA receptor stimulation and subsequent activation of calmodulin-dependent kinases II and IV, protein kinase C and the calcium-cAMP response element binding protein (CREB) (Arumugam et al., 2005). In experiments using primary cultures of hypothalamic neurons, inhibition of NMDA receptor signaling with the antagonist DL-2-amino-5-phosphonovalerate (AP5) did not affect the initial increase in gap junction coupling that occurs within the first 16 days in vitro, but AP5 nearly abolished uncoupling that typically occurs over the following two weeks. Similar results were obtained using neuronal cultures from mice lacking the NR-1 subunit of the
NMIA receptor. In vivo studies were also performed with newborn rats injected daily with the NMDA antagonist dizocilpine (MK-801). NMDA receptor blockade again had no effect on the initial increase in gap junction coupling, but inhibited uncoupling in 4-week-old rats treated with MK-801. Thus the in vivo results were consistent with in vitro experiments demonstrating that developmental uncoupling of electrical synapses containing Cx36 in immature neurons requires NMDA receptor activation (Arumugam et al., 2005).

5. Interactions between pannexin1 and Kβ3

Pannexins (Px) represent a novel group of putative gap junction proteins that are unrelated to Cx and are expressed by both vertebrate and invertebrate species (Bruzzone et al., 2003; Baranova et al., 2004; Hervé et al., 2005; Barbe et al., 2006). Three Px genes have been identified in mammals. Although direct proof that pannexins form functional intercellular junctions is lacking, functional hemichannels and intercellular channels have been detected and characterized in Xenopus oocytes expressing Px mRNA (Bruzzone et al., 2005; Vogt et al., 2005; Barbe et al., 2006). Moreover, Px1 and Px2 have been shown to form heteromeric hemichannels when expressed in oocytes (Bruzzone et al., 2005). Unlike Cx, homomeric and heteromeric Px channels are not gated by extracellular Ca²⁺. However, Px1 and Px1/Px2 channels are sensitive to two commonly used gap junction blockers, carbenoxolone and β-glycyrrhetinic acid. In contrast, Px channels are relatively insensitive to flufenamic acid, a compound known to block Cl⁻ channels as well as connexin hemichannels and intercellular channels (Bruzzone et al., 2005). Recently, Kβ3, an auxiliary subunit known to interact with and modulate voltage-activated K⁺ channels was identified as a binding partner of Px1 (Bunse et al., 2005). Although this result on its own is not sufficient to prove that the interaction is physiologically important, further studies confirmed the colocalization of Px1 and Kβ3 in Neuro2A cells expressing these proteins using confocal microscopy. Co-localization of both Px1 and Kβ3 in the plasma membrane suggests that a functional interaction exists between these proteins and the interesting possibility that auxiliary subunits typically associated with other plasma membrane ion channels may have chaperone or regulatory interactions with gap junction channels.

6. Summary and conclusions

The CFTR-dependent opening of Cx45-containing gap junction channels observed in pancreatic duct cells may favor the transjunctional flux of cAMP. This mechanism, in turn, may thereby optimize pancreatic duct fluid secretion in a heterogeneous population of CFTR-expressing cells. The possibility that cAMP diffuses through gap junctions to regulate CFTR activity in neighboring cells has been recently verified (Qu and Dahl, 2002). Defects in this mechanism, due to reduced CFTR function, might contribute to the abnormal pancreatic juice composition that is observed in CF.

The significance of the TNF-α-dependent closure of Cx43-containing gap junction channels observed in normal airway cell lines expressing CFTR is unclear. So far, there is no evidence that Cx43 is expressed by the normal airway epithelium, although this connexin is present in de-differentiated airway cells (Brezillon et al., 1997; Chanson and Suter, 2001). According to Huang et al. (2003), Src activation is defective in CF epithelial cell lines. In addition, LPA, which is known to activate c-Src (Giepmans et al., 2001), markedly decreased dye coupling in primary cultures of de-differentiated non-CF airway cells and tyrphostin47, an inhibitor of Src tyrosine kinases, prevented the uncoupling normally induced by TNF-α (Huang et al., 2003). These results suggest that the absence of CFTR from the plasma membrane in CF epithelial cells may compromise the regulation and/or the stability of the Src proteome. Possibly, the CFTR proteome is required for proper transcriptional activity of proteins involved in c-Src regulation. In this case, CFTR-interacting proteins might serve as transducers for CFTR signaling. Alternatively, CFTR may interact with cAMP- and c-Src-signaling complexes. This may result in altered Src-dependent regulation of not only gap junction connectivity but also of other target proteins involved in various cell functions, including cytokine production, mucus secretion and epithelium repair after injury. Whether there is a link between these defects and Cx43 in CF is an interesting question for future studies.

The interactions between Cx45.6 and MIP/AQP0 in lens did not appear to affect intercellular communication but was dependent on the state of differentiation of the fiber cell. What this suggests is
that the Cx45.6 may somehow be involved in a function of MIP/AQP0 that does not directly involve water transport, but may influence the process of cell differentiation (Yu et al., 2004, 2005). In contrast, decreases in AQP4 expression in mouse astrocytes specifically reduces gap junction connectivity mediated by Cx43. Although the exact molecular nature of this interaction has yet to be defined, the net consequence may be to coordinate the intercellular movement of ions and water between astrocytes, which may have important consequences on local extracellular electrolyte concentrations at various locations within the brain (Nicchia et al., 2005).

A functional interaction between Cx35/36 and glutamate receptors has been shown to be essential for activity dependent short-term plasticity associated with electrotonic synaptic transmission in the vertebrate nervous system (Pereda et al., 2003a, b). A direct molecular interaction between NMDA receptors and Cx35/36 has not been established, thus it seems likely that downstream signaling events evoked by glutamate are responsible for the observed increase in gap junction conductance and electrotonic synaptic transmission. This speculation is consistent with recent developmental studies of hypothalamic neurons that demonstrated down regulation of Cx36 expression and electrical coupling between neurons following NMDA receptor activation. This effect required activation of multiple enzymes involved in protein phosphorylation and nuclear transcription factors that modulate transcriptional activity of the cell (Arumugam et al., 2005). Although the developmental and plasticity responses to NMDA receptor activation are distinct, the results demonstrate that glutamate signaling fundamentally alters intercellular communication between neurons in the CNS.

Finally, recent studies describing a possible functional interaction between pannexin1 and the $K_v \beta_3$ auxiliary subunit suggest the interesting possibility that pannexins or perhaps certain Cx form associations with auxiliary subunits of plasma membrane ion channels or transporters. These interactions may be important for trafficking and localization of gap junction subunits or may modulate conduction properties or regulation of gap junctions (Bunse et al., 2005). Future studies should help to determine the importance of these interactions and shed light on the ways in which intercellular communication is affected.

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References


