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Review

Gap junctions and connexin-interacting proteins

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Abstract

Gap junctions form channels between adjacent cells. The core proteins of these channels are the connexins. Regulation of gap junction communication (GJC) can be modulated by connexin-associating proteins, such as regulatory protein phosphatases and protein kinases, of which c-Src is the best-studied. Structural proteins, notably zona occludens-1 (ZO-1) and microtubules, have been found recently at gap junctions. Along with the expansion of the list of connexin-associating proteins, reports have appeared that suggest that connexins might have additional roles in addition to their channel function, such as transcriptional and cytoskeletal regulation. Here, gap junction interacting proteins are reviewed and their function is addressed. The striking similarity of proteins present at the cytoplasmic face of tight junctions, adherens junctions and gap junctions and their possible role in gene transcription and cytoskeletal anchorage is highlighted.

Keywords: Connexin; Binding protein; ZO-1; Cytoskeleton; Microtubule; Tight junction; Adherens junction; Gene transcription

1. Introduction

Desmosomes, tight junctions, adherens junctions and gap junctions were originally identified by their morphological appearance in electron microscopy (EM). During the last two decades, the molecules that contribute to the structure, function and regulation of cell–cell junctions are beginning to be identified. Interestingly, evidence is accumulating that cell–cell junctions might initiate signals that modulate gene transcription and growth control. Identification of proteins interacting with the junctional proteins is crucial for unravelling possible additional roles of cell–cell contacts. Proteins associated with desmosomes [1,2], tight junctions [3–5] and adherens junctions [6,7] have been reviewed recently. The current review summarizes proteins reported to interact at gap junctions. The focus will be on the most widely expressed and best-studied connexin originally identified in heart: Connexin43 (Cx43). Strikingly, several of the proteins identified at gap junctions had been localised at adherens junctions and tight junctions years before. Shared characteristic between the cell–cell junctions will be discussed.

2. The gap junction: only a channel?

2.1. Architecture of gap junctions

The pore size of gap junction channels allows diffusion of molecules of less than ~1000 Da. One gap junction channel consists of multimers of the four-transmembrane core protein, the ‘connexin’, of which 20 members have been identified in human [8]. Six connexins form one connexon, which transverses the plasma membrane of one cell and docks with a connexon of the neighboring cell, thereby creating an extracellular gap (reviewed in Refs. [9–12]). The three-dimensional channel structure for C-terminal truncated Cx43 revealed that opposing connexons are staggered by 30° and are tightly packed in the intercellular gap [13,14]. Inhibition of gap junction communication (GJC) might be achieved by a twist of the connexons, like in a diaphragm (reviewed in Ref. [9]). Alternatively, the channel might be blocked by other molecules or by the connexin tail, the so-called “ball-and-chain” model [15].

The large number of connexin sub-types allows a fine-tuned regulation of expression, gating, and channel pore size. At the protein level, growth factors and second messengers can regulate gating of gap junctions in a connexin-specific manner. Selectivity of pore size and gap

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junction regulation by growth factors are illustrated in the retina model (Section 3.1).

2.2. Physiological importance of gap junctions

Numerous processes are driven by GJC, such as (i) rapid transmission of action potentials in heart and in neuronal tissue via so-called electrical synapses [16,17]. (ii) Diffusion of metabolites and nutrients, such as nucleotides and glucose [18], which depends on the channel type: Cx32 channels are more permeable to adenosine than Cx43-channels. ATP, however, passes more readily through Cx43 channels [19]. (iii) Diffusion of second messengers, such as Ca^{2+}, inositol-trisphosphate (IP_3) and cyclic nucleotides [20] (reviewed in Ref. [10]) might be involved in induction of apoptosis [21], gene transcription [22] and growth control [23].

In Cx43 knock-out mice, deregulation of neural crest cell migration during embryonic development results in heart malformation (reviewed in Ref. [24]). Deregulation of Cx43 has been studied in more molecular detail in the cancer research area. The phenotype of certain transformed cells can be reversed in a Cx43-dependent manner when they are co-cultured with their non-transformed counterparts [25] (reviewed in Ref. [26]). This might involve apoptotic signals that can be transferred between these cells via gap junctions the so-called ‘‘bystander effect‘‘ [21]. Moreover, oncogenic v-Src has been shown to rapidly and reversibly inhibit GJC [27] as is further described in Section 3.3.1.

2.3. The Cx43-tail tale

Initially, all effects of connexins were attributed to direct cell-to-cell diffusion. However, some of the connexin functions seem to occur unrelated to channel function. Cx43 and Cx32 mutants lacking the C-terminal tail expressed in transformed cells restored GJC and inhibit proliferation. The full-length proteins did induce GJC as well, but did not reduce the growth rate [28]. Moreover, Cx43 has also been shown to inhibit proliferation when GJC was blocked by pharmacological inhibitors, or channel formation is prevented [29]. In addition, Cx43 point-mutants that did block GJC still reduced cell growth, and expression of the C-terminal tail alone gave similar results (see below). A Cx43 mutant that does not form gap junctions, since it is not properly localized to the plasma membrane, has been shown to suppress cell growth [30].

These studies raise the possibility that connexin C-terminal tails, and that of Cx43 in particular, can modulate gene expression via binding proteins. The overexpressed Cx43CT localises to the nucleus [31] and does inhibit cell growth [29,31]. So far, no evidence of an endogenous cleavage of the tail has been reported, but the C-terminal tail recently has been related to cell-cycle control. Cx43 overexpression can inhibit cell proliferation, accompanied by the decreased stability of Skp2, a protein involved in cell-cycle regulation. Inhibition of proliferation was found with full-length Cx43, but no effect was seen when mutants lacking the C-terminal tail were expressed [32]. These studies suggest that Cx43 can modulate the cellular genetic program via its C-terminal tail.

3. Connexin-interacting proteins

A complete understanding of connexin and gap junction function can only be achieved when the proteins that are present at gap junctions are identified. Several proteins might be involved in connexin transport, gap junction formation and gating, while others might be involved in novel functions of gap junctions. When studying protein–protein interactions at subcellular structures, the results of co-immunoprecipitation or co-localisation alone should not be interpreted as direct protein–protein interactions (see also Table 1, Figs. 1 and 2).

3.1. Other connexins

Trivial but important: interactions between the different types of connexins define channel characteristics. Combination of different connexins can result in formation of homomeric and heteromeric connexons (reviewed in Refs. [9,10]). Furthermore, the composition of two docking connexons can be different. The differences in channel pore size and regulation that can be achieved are impressively illustrated in the retina. Gap junctions in amacrine cells enable diffusion of tracers of 286 and 442 Da between cells (homologous coupling). However, when these cells are coupled to cone bipolar cells that express another type of connexin (heterologous coupling), only diffusion of the 286-Da tracer is found. Furthermore, homologous coupling is sensitive to dopaminergic stimulation and rise in cAMP levels, while heterologous coupling is affected by cGMP and nitric oxide [33]. This model shows that differences in connexin expression determine gating and channel size in a complex manner.

3.2. Proteins identified at other cell–cell junctions

3.2.1. Zona occludens-1 protein

Zona occludens-1 protein (ZO-1) has originally been identified at tight junctions and later at adherens junctions (Section 4). Interaction of ZO-1 with multiple connexins is now well characterized (Table 1). The first two reports describing the Cx43-ZO-1 interaction showed that the interaction occurs at gap junctions. However, sequence of the Cx43 tail did not match the consensus sequence known at that time for PDZ binding [34]. Yet, the interaction was shown to be a classical juxtamembrane protein with PDZ-motif/PDZ binding (Fig. 1) [35]. Removal of the C-terminal isoleucine or extending the tail with different tags complete-

Table 1
Connexin-interacting proteins

Protein	Methods	aa connexin		Partner	References
Cx43					
ZO-1	FWB[a], Y2H, PD, IP[b], IF[b]	379–382	–Ile	PDZ2	[35,37,41,66,125]
v-Src	PD, IP, P	247, 265	Tyr	kinase	[56–59]
		265	Tyr-p	SH2	[58,59]
		274–283	PxxP	SH3	[58,59]
c-Src	PD, IP, P	265	Tyr	kinase	[36,63,65,66]
		265	Tyr-p	SH2	[36,63,65,66]
PKA[c]	P	364, 365, 368, 369, 373	Ser	kinase	[75,76]
PKCs	P, IP[b], IF[b]	368, 372	Ser	kinase	[71–74,126]
PKG	P	257 (rat)	Ser	kinase	[77]
MAPK	P	255, 279, 282	Ser	kinase	[127]
Cdc2	P	255	Ser	kinase	[85,86]
CK1	P, IP	325, 328, 330	Ser	kinase	[87]
RPTPμ	IP[b]	265[c]	Tyr-p	phosphatase	[70]
β-Catenin	IP[b]				[49]
α-Catenin	EM[b]				[47]
Cadherin	EM[b]				[47]
p120[ctn]	IF[b]				[50]
NOV/CNN	IF				[108]
Caveolin	PD, IP, IF[b], CS				[104]
α/β-Tubulin, MTs	PD, IF[b], EM[b], CS[a]	234–262	K,G,V,R,P		[41,97]
unknown		282–289	xPPxYxxO		[128]
Cx32					
Calmodulin	IF				[106]
Occludin	EM[b], IP[b]				[53,54]
Claudin	EM[b], IP[b]				[54]
Cx49					
CK1	P, IP			kinase	[88,89]
Cx50					
CK2	P	363	Ser	kinase	[90]
Caveolin associated connexins					
Cx43, 32, 36, 46	CS				[104]
ZO-1 interacting connexins					
Cx43	FWB[a], Y2H, PD, IP[b], IF[b]	C-terminal	–Ile	PDZ2	[35,37,41,66,125]
Cx45	Y2H, IP, IF	C-terminal	–Ile	PDZ2	[38,39]
Cx46	PD, IP, IF	C-terminal	–Ile	PDZ2	[129]
Cx50	PD	C-terminal	–Val	PDZ2	[129]
Cx31.9	PD	C-terminal	–Ile	PDZ2	[130]
Cx36	PD, IP, IF, EM	C-terminal	–Val	PDZ1	[40]

Direct associations with ZO-1 and Src are well-established. Cx43 is a substrate of several other kinases, but direct interaction with most kinases has not been found. Other interactions do warrant follow-up studies since they only just emerged, are less well documented, might be indirect or are found in overexpression systems. See text for further nuances. Abbreviations: aa: amino acids, FWB: far Western blot, Y2H: yeast two-hybrid, PD: GST protein pull-down, IP: immunoprecipitation, IF: immunofluorescence, EM: (immuno)-electron-microscopy, P: phosphorylation state, CS: in vitro co-sedimentation assay.

[a] Showing direct interaction.

[b] Includes experiments with endogenous proteins only, without inhibitors.

[c] Putative.

ly abolished the binding (see also Ref. [36]). However, the interaction between ZO-1 and Cx43 has also been demonstrated using a C-terminal flag-tagged Cx43 to co-immunoprecipitate ZO-1 [37]. The discrepancy in these findings might be explained by the different cell-types that were used (COS7 and HEK293 cells, respectively). The latter cell type expresses moderate levels of endogenous Cx43 that might bridge Cx43-flag and ZO-1.

Other connexins have now been found to bind to ZO-1 as well (Table 1). Interestingly, a reciprocal two hybrid assay

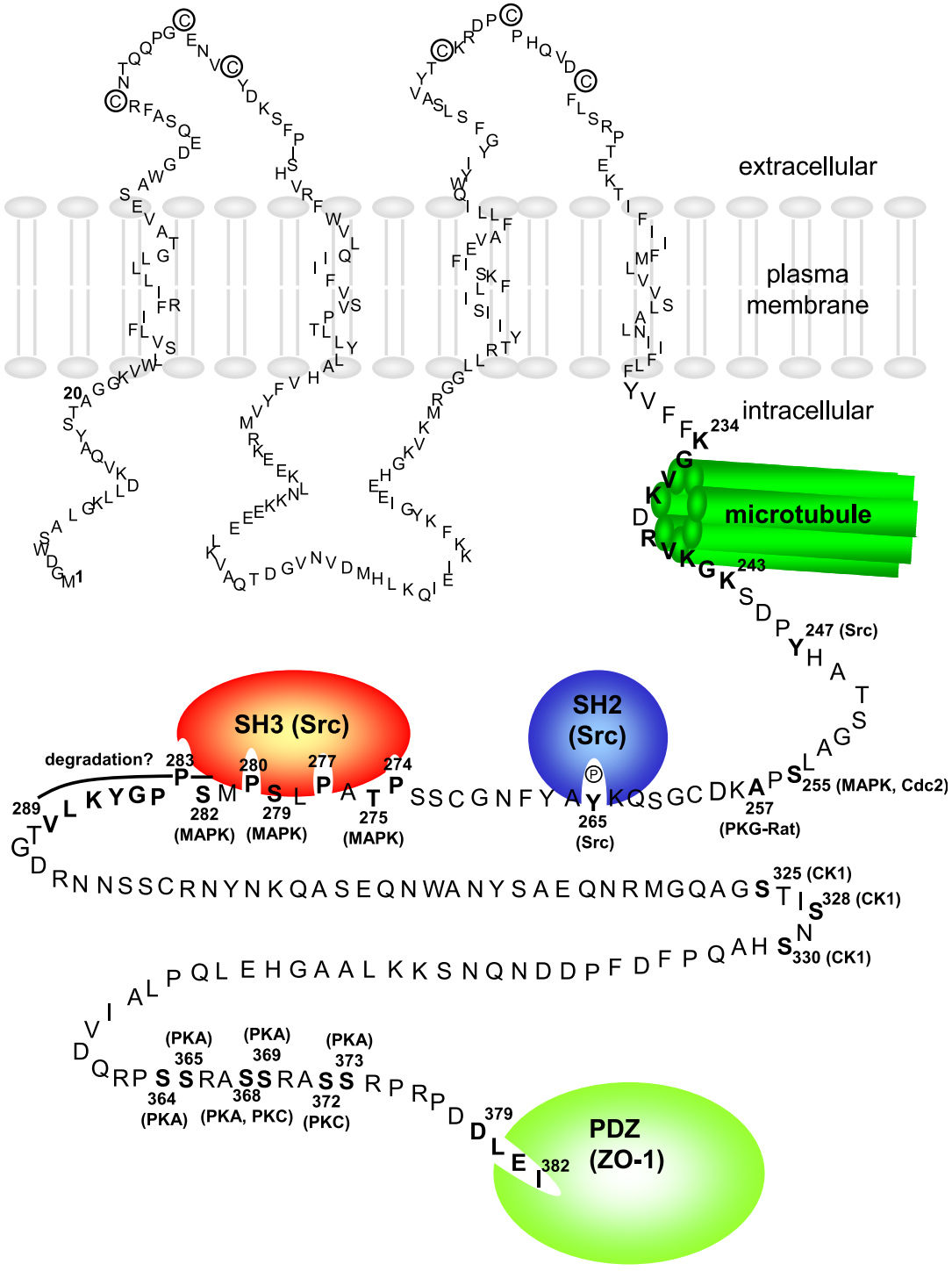

Fig. 1. Human Cx43 amino acid sequence and consensus interacting protein modules. Conserved cysteines in the extracellular gap are encircled; proposed regulatory sites are in bold. For a complete overview of modular protein interaction domains and consensus sequences, see Ref. [124]. Src homology 2 domain (SH2). SH2 domains characteristically bind phospho-tyrosine residues in a hydrophobic pocket. An additional pocket confers substrate specificity by binding amino acids more C-terminally located. Note that Tyr265 of Cx43 is target of Src phosphorylation. Src homology 3 domain (SH3). Aliphatic proline residues of the substrate located on one side of an α-helix bind separately to hydrophobic pockets in the SH3 domain. The flanking region determines binding specificity. PSD95/disc large/ZO-1 homology domain (PDZ). PDZ domains form a hydrophobic pocket that binds the very C-terminal residues (-x-S/T/V/I/L-x-V/I/L-COOH) of their target proteins typically transmembrane proteins [34]. *Microtubules*. Microtubule binding regions are often enriched in K, V, G, P and R. See Table 1 and text for further details.

using PDZ domains of ZO-1 as bait showed Cx45 interaction with ZO-1 and ZO-3, but not ZO-2 [38]. This confirmed the ZO-1/Cx45 interaction found using biochemical techniques [39]. Whenever binding sites were mapped, the C-terminal amino acids of connexins and the second PDZ domain of ZO-1 were found to mediate the interaction. Recently however, Cx36 has been found to bind exclusively to the first PDZ domain of ZO-1 [40]. Based on their C-terminal amino acid sequence, all human connexins, except Cx30, 30.3, 31.3, 32 and 62, are potential ZO-1 binders.

C-terminal tagging or mutation of Cx43 abolishes ZO-1 interaction [35,41], but truncated [14] or tagged [42,43] connexins form gap junction plaques. Therefore, functional involvement of ZO-1 in gap junction formation is unlikely. However, the plaques formed with tagged Cx43 seem to be larger than with endogenous Cx43, which might point to a function of ZO-1 in gap junction turnover [42–44]. Further experimentation is needed to test this model.

Previously, we postulated a function for ZO-1 in assembly of the signaling proteins at gap junctions [35]. However, all candidates we now have tested, including several PKCs, PLC, G proteins, G protein-coupled receptors, but also β-catenin and p120^{ctn}, could not be found in pull-downs with the SH3 or PDZ domains of ZO-1 (unpublished results). Whether gap junctional ZO-1 might serve a role in cytoskeletal anchorage and sequestering of transcription factors as has been postulated for adherens junctions and tight junctions is discussed in Sections 3.5 and 4.

3.2.2. Cadherins, β-catenin and p120^{ctn}

Cadherin-mediated cell–cell adhesion (Fig. 2, Section 4.1) is a prerequisite for formation of gap junctions. Upregulation of E-cadherin-dependent cell–cell contacts has been shown to increase GJC [45]. Furthermore, anti-N-cadherin antibodies prevent both adherens junctions formation and GJC [46]. E-Cadherin and α-catenin are co-localized with newly formed gap junctions, which might reflect an adherens junction focus that primes gap junction formation [47]. Moreover, N-cadherin has been shown to co-localize with Cx43 in cardiac myocytes [48]. In the same cell-type, β-catenin has been found to co-localize and co-immunoprecipitate with Cx43 following Wnt expression [49]. Another catenin, p120^{ctn}, has been found to co-localize not only with cadherins, but also with Cx43 [50].

Besides its role in adherens junctions, β-catenin acts as a transcriptional co-factor downstream of the Wnt signaling

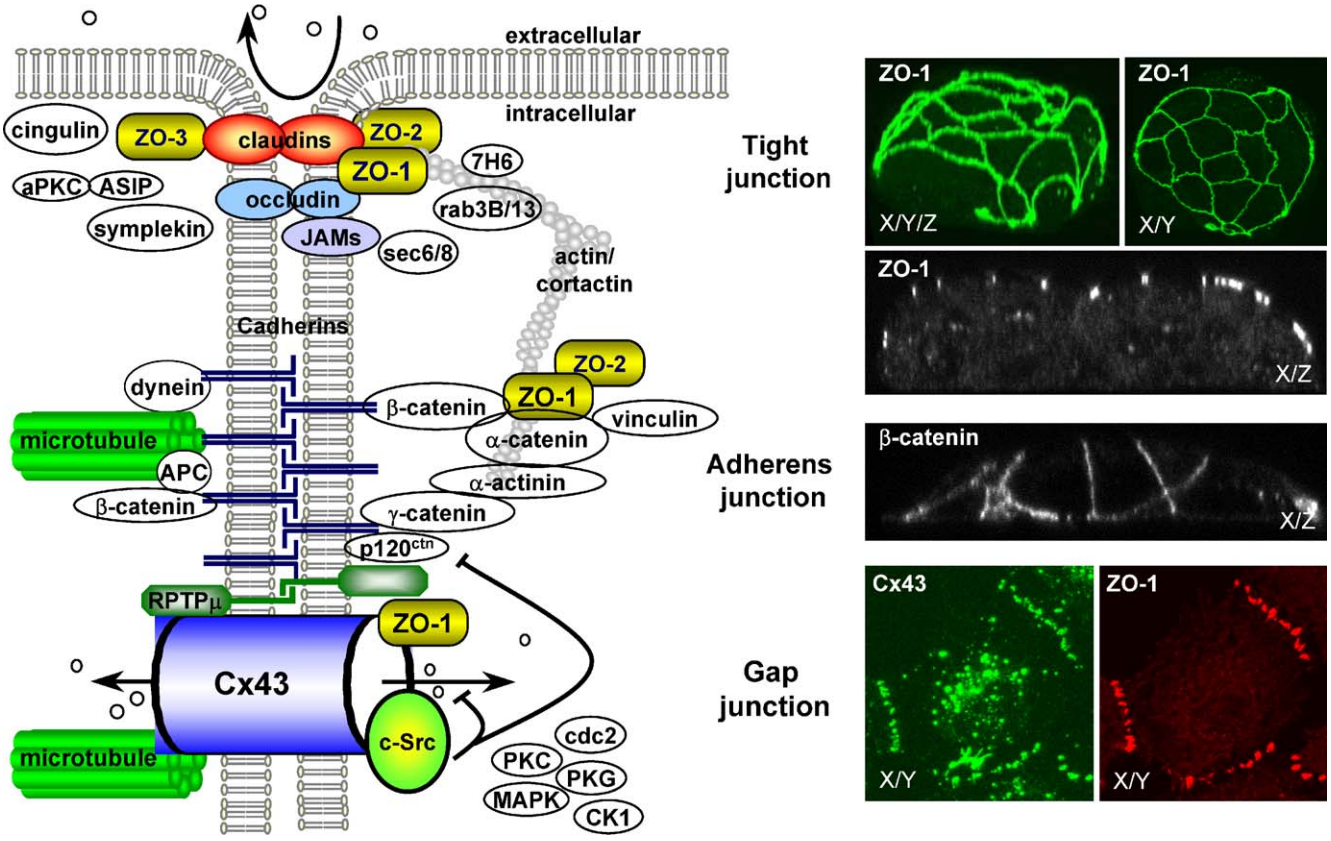

Fig. 2. Molecular and cellular organisation of cell–cell junctions. Left: Cx43-associating proteins are depicted. See recent reviews for a complete picture of all known proteins associated with adherens [6] and tight junctions [4,5]. See text for details. Right: Confocal scanning light microscopy. Tight junctions form a network, which is only present in cell–cell contacts that face the apical contact sites of cells (20 MDCK cells; a three dimensional reconstruction (left; courtesy of Lauran Oomen), a projection (right) and an X/Z section are shown). Adherens junctions zip cells together (MDCK cells; middle panel). Gap junction plaques show a typical punctate staining (Rat-1 fibroblast; bottom panel).

pathway. Although controversial, cadherins have been proposed to sequester β-catenin at adherens junctions and thereby modulate Wnt-mediated gene transcription [51] (reviewed in Ref. [52]). In analogy with this model, inhibition of lithium-induced Wnt signaling found in Cx43 overexpressing cells has been suggested to be mediated by sequestration β-catenin at gap junctions [49]. Despite lack of evidence of a direct interaction between connexins and β-catenin to date, the physical interactions found so far and a possible function in GJC-independent regulation of gene expression by Cx43 by sequestering transcriptional regulators are noteworthy and will be further discussed in Section 4.

3.2.3. Claudins and occludin

Claudins and occludin are core proteins of tight junctions (Fig. 2, see also Section 4.2). Occludin has been reported to co-immunoprecipitate with overexpressed Cx32 in one clone, but not in another [53]. At the ultrastructural level, EM convincingly showed distinct tight junction strands surrounding gap junction plaques [53]. In a follow-up study, Cx32 was not only found in immunoprecipitations of occludin, but also of ZO-1 and claudins [54]. Site-directed mutagenesis and direct protein–protein interaction assays should answer whether these core proteins are connected directly or entire gap junction plaques are co-precipitated with tight junction strands.

3.3. Tyrosine kinases and phosphatases

Pharmacological inhibitors of kinases and phoshatases have been useful tools to determine which kinases play an (in)direct role in connexin phosphorylation. Furthermore, inactive kinases and site-directed mutagenesis of connexins has lead to progress in mapping of phosphorylation sites.

3.3.1. Src

Before the first gap junction proteins were cloned, the viral Src oncoprotein was already known to shut down GJC [55]. Once cloned, Cx43 was soon found to be a v-Src substrate [56,57]. Mutation of the putative v-Src phosphorylation site results in lack of gap junction closure by v-Src in Xenopus oocytes [57]. Further studies showed that phosphorylated Tyr265 in Cx43 forms a docking site for the SH2 domain of v-Src and that the SH3 domain of v-Src can bind to a proline rich stretch in Cx43 [58] (Fig. 1). In addition, Tyr247 can be phosphorylated by v-Src [59]. A model was proposed in which (i) v-Src binds via a SH3 domain/proline-rich motif association; (ii) Src than phosphorylates Cx43, mainly on Tyr265; and (iii) subsequent docking of the SH2 domain of Src to Cx43Tyr265 increases affinity and positions Src for (iv) Tyr247 phosphorylation leading to channel closure.

Following the work on oncogenic v-Src, its normal cellular counterpart c-Src has been shown to inhibit Cx43-based GJC downstream of receptor stimulation as well. c-Src activation is used by several receptor-families to finally inhibit GJC, namely G protein-coupled receptors, notably those for lysophosphatidic acid (LPA), endothelin-1 and thrombin [60,61,62], tumour necrosis factor-α (TNFα [63]) and endotoxin, which may stimulate the toll-like receptors [64]. These studies show that ligand-induced gap junction closure is inhibited by tyrosine kinase inhibitors, and in particular by Src kinase inhibitors. Furthermore, inhibition of GJC correlates with Src activation and tyrosine phosphorylation of Cx43, while gap junctions in Src deficient cells do not close in response to ligand-stimulation. In vivo data showed a correlation between Cx43 tyrosine phosphorylation and Src activity in cardiomyopathic heart [65]. This finding further suggests that c-Src might be an important (patho)physiological regulator of Cx43. In vitro models revealed that the SH2 and SH3 are involved in this interaction (Fig. 1), and Src binding to Cx43 excludes the C-terminal tail for ZO-1 binding [66]. Cx43Tyr265 was shown to be phosphorylated by Src and phospho-Tyr265 was found to be crucial for the co-immunoprecipitation of Cx43 and Src [36,66]. The inability of Src to phosphorylate and associate with Cx43Y265F leads to a rescue of Src-induced gap junction closure [36,66]. Moreover, Cx43Y265-F;Y247F gap junctions are no longer regulated by TNF-α [63]. Taken together, these data show that certain ligands utilize c-Src to regulate GJC via a mechanism possibly similar to that described above for v-Src.

3.3.2. RPTPμ

Inhibition of tyrosine phosphatases by pervanadate results in Cx43 tyrosine phosphorylation and closure of gap junctions [61,67,68]. Interestingly, Cx45 gap junctions have also been shown to be inhibited by pervanadate [69]. Tyrosine phosphorylation of the Cx43 C-terminal tail might trigger the closure, similar to Src action. Recently, we showed that the phosphatase domain of receptor protein tyrosine phosphatase μ (RPTPμ) brings down overexpressed Cx43. Furthermore, endogenous RPTPμ was co-immunoprecipitated with Cx43 [70]. These data favor a model in which RPTPμ can counteract Src phosphorylation at gap junctions, keeping Cx43 in the non-phosphotyrosine state and thereby prevent channel closure. However, further studies are needed to substantiate this notion.

3.4. Serine/threonine kinases and phosphatases

Serine and threonine kinases that phosphorylate Cx43 (Fig. 1, Table 1) are less well documented to form a complex with Cx43. These kinases will therefore be described only briefly.

3.4.1. PKC/PKA/PKG

The best studied of these three kinases in gap junction regulation is the one activated by phorbol ester: protein kinase C (PKC). Phorbol ester treatment of cells can result in dramatic activation of multiple PKC isotypes and phos-

phorylation of several substrates, but a direct effect of PKC on gap junctions has been difficult to address. An elegant study combining in vivo phosphopeptide mapping and site-directed mutagenesis showed that PKC acts on Ser368 of Cx43 (see Table 1 and Fig. 1), resulting in inhibition of GJC (see Ref. [71] and references therein). Interestingly, PKCε, which has been implicated in inhibition of GJC downstream of the fibroblast growth factor-2, has been reported to co-immunoprecipitate and co-localize with Cx43 [72] in cardiomyocytes. Similar results have been described for the PKCγ isotype acting downstream of the insulin-like growth factor receptor-I [73]. However, lack of any effect of PKC on GJC [61] or even an upregulation of GJC by PKCα paralleled with Cx43 phosphorylation [74] have also been described. The seemingly controversial effects of PKCs on Cx43 and GJC likely reflect the complexity of channel regulation. The convergence of several signal transduction pathways that impinge on Cx43 turnover, transport and assembly and gating of gap junctions dictate GJC status.

cAMP-dependent protein kinase (PKA) has been shown to upregulate Cx43 assembly. Although Ser364 is critically involved in the enhanced assembly, it remains unclear if PKA directly phosphorylates Cx43 on this residue. A negative charge on this site, which might be through phosphorylation of Ser364 by another kinase, seems to be critical for PKA-induced assembly of gap junctions [75] and references therein. Activation of PKA by ligand stimulation and subsequent phosphopeptide mapping of point-mutated Cx43 has implicated phosphorylation of serines 365, 368, 369 and 373 downstream of PKA. GJC was completely reduced when the quadruple mutant was expressed [76].

cGMP-dependent protein kinase (PKG) has been shown to phosphorylate rat Cx43, which is correlated with a decrease of GJC [77]. However, the processes in which PKG might be involved to reduce gating remain to be elucidated.

3.4.2. MAPK

Mitogen-activated protein kinase (MAPK)-mediated Cx43 serine phosphorylation induced by LPA [78] and epidermal growth factor (EGF) [79] has been postulated to control inhibition of Cx43-based GJC. In epithelial cells, inhibition of GJC and MAPK activation by EGF correlated well in a time- and dose-dependent manner [79]. Stimulation of epithelial cells with platelet-derived growth factor (PDGF) leads to a rapid, but transient inhibition of GJC, accompanied by an increase in Cx43 phosphorylation [80], mediated by MAPK and PKC. In a more recent study, however, MAPK-mediated Cx43 phosphorylation induced by PDGF did not result in inhibition of GJC [81]. Moreover, kinetics of MAPK activation correlated with Cx43 phosphorylation in EGF- or phorbol ester-treated cells. However, the effects on GJC were opposite: EGF increased GJC, whereas phorbol ester inhibited GJC [82]. Increased GJC has also been shown after sustained MAPK activation [83]. Moreover, v-Src activates MAPK, but blocking MAPK activation does not block Src-induced gap junction closure [15]. Furthermore, long-term treatment of cultured cardiomyocytes with endothelin-I or angiotensin-II results in a MAPK-dependent upregulation of Cx43 and increase in GJC [84].

In conclusion, MAPK-mediated phosphorylation of Cx43 is well established, but can have opposite effects on GJC.

3.4.3. Cdc2

Cdc2 is indispensable for cell-cycle progression. Direct phosphorylation of Cx43 by Cdc2 could be detected in vitro on peptides with the putative target residue Cx43Ser255 or using the entire C-terminal Cx43 tail. Cx43 was phosphorylated on Ser255 in a Cdc2-dependent manner, but also on other sites as well, which might reflect activation of kinases downstream of Cdc2 [85,86]. Cdc2-mediated connexin phosphorylation might reflect plasma membrane deprivation of gap junctions during mitosis.

3.4.4. CK1 and CK2

Cx43 gap junction assembly might be affected by phosphorylation by casein kinase 1 (CK1). Target residue(s) are the serines between 320 and 345, with Ser325, 328 and 330 being the candidate serines for CK1 action. CK1 has been found to co-immunoprecipitate with Cx43. Inhibition of CK1 reduced Cx43 phosphorylation concomitant with a decrease of plasma membrane targeting of Cx43 [87]. However, truncation mutants lacking these sites have been shown to form gap junctions [14]. Further studies should reveal the exact target sites of CK1 and to what extent CK1 is a major player in assembly of connexins. In addition to Cx43, Cx49 has been reported to be a CK1 substrate, but the effect of phosphorylation remains unknown [88,89]. Casein kinase 2 (CK2) is an upstream kinase for the chicken homologue of human Cx50. Cx50Ser363 is phosphorylated by CK2. The Cx50Ser363Ala mutant is more stable, suggesting that CK2 phosphorylation might target Cx50 for degradation [90].

3.4.5. Phosphatases

GJC is affected by several inhibitors of serine and threonine phosphatases (reviewed in Ref. [91]). However, the identity of particular serine/threonine phosphatases and their effect on connexins remain to be identified. To date, no physical interactions between connexins and this group of phosphatases have been reported.

Taken together, serine phosphorylation may affect several properties of Cx43: its transport to the plasma membrane [92], assembly into connexons and gap junctions [93], internalisation [85], its degradation [94], disassembly [95] and gating. For more details on serine/threonine kinases acting on connexins, the reader is referred to two recent comprehensive reviews [91,96].

3.5. Cytoskeletal proteins

3.5.1. Microtubules and tubulin

Recently, we found that Cx43 directly binds to α- and β-tubulin and that microtubules at the cell-periphery co-localize with Cx43-based gap junctions [41,97]. The 35 juxtamembrane amino acids of the C-terminal tail were found to be necessary and sufficient for this interaction (Fig. 1). Inhibition of GJC by endothelin was not affected by microtubule disassembly [97]. In contacted cells, microtubule dynamics is suppressed [98]. It has been postulated that cell–cell contact promotes the activity of an as-yet-unidentified ''plus-end'' capping protein at the cell periphery, that might be adherens junction or gap junction proteins. Moreover, cadherins may regulate microtubule stability [99], and dynein links microtubules via β-catenin to adherens junctions [100]. Another protein that might link microtubules to adherens junctions via β-catenin is adenomatous polyposis coli protein (APC) [101]. These studies suggest that gap junctions and adherens junction might function as microtubule-anchoring points (see also Section 4).

3.5.2. Actin and α-spectrin?

Actin is linked to adherens junctions and tight junctions (Section 4, Fig. 2) via α-catenin and ZO-1 [7,102]. Therefore, it is tempting to speculate that ZO-1 might also link actin to gap junctions. Another cytoskeletal protein linked to tight junctions by ZO-1, is spectrin [103]. In studies over-expressing c-terminal tagged Cx43 and ZO-1, spectrin has been reported to co-immunoprecipitate with gap junctional ZO-1 [37]. However, further research is needed to address the cell biological relevance of this interaction.

3.6. Other proteins

3.6.1. Caveolin

Recently, Cx43 was found to bind two distinct domains of caveolin, the marker of the caveolae lipid rafts. Besides Cx43, Cx32, Cx36 and Cx46 were found to co-sediment in the same fraction as caveolin, whereas Cx26 and Cx50 were not [104]. This suggests that multiple connexins might localize to lipid rafts, but the function of this interaction remains to be established.

3.6.2. Calmodulin

Calmodulin was found to directly bind to proteins immunoprecipitated with a crude anti-gap junction antibody [105]. Recently, gating of Cx32 gap junctions was shown to be affected by calmodulin mutants in Xenopus oocytes. Moreover, overexpressed Cx32 co-localized with calmodulin [106]. These data, together with earlier work (see Ref. [96,106] and references therein) suggest that calmodulin can regulate gap junction gating. Whether this involves a direct interaction with Cx32 warrants further investigation.

3.6.3. NOV/CCN3

NOV/CCN3 is a secreted extracellular matrix protein with growth suppressive effects and has been implicated in angiogenesis [107]. NOV has been found to be upregulated in Cx43 overexpressing cells using micro-array. Strikingly, NOV co-localizes with Cx43 gap junctions [108]. Further developments should reveal the significance and function of NOV at gap junctions.

4. Connexin-associated proteins at other cell–cell junctions

Several proteins described recently identified at gap junctions were identified before at tight junctions and/or adherens junctions. This leads to unexpected shared characteristics between these junctions, which had been so distinctly grouped on EM characteristics and ''primary function''. Putative novel functions in cytoskeletal anchorage and transcriptional regulation that might be shared between adherens, tight and gap junctions are discussed.

4.1. Adherens junctions

Adherens junctions zip cells together, and thereby maintain cell and tissue polarity (Fig. 2). Furthermore, these junctions anchor the cytoskeleton to the plasma membrane [6,109]. The structural proteins of adherens junctions are the transmembrane proteins cadherins, which form homodimers in the intercellular space in a calcium-dependent manner. The short intracellular domain of cadherin can bind $p120^{ctn}$, β-catenin and γ-catenin (plakoglobin). β-Catenin on its turn binds to α-catenin that recruits ZO-1 [102,110,111], vinculin and α-actinin. The major proteins linking actin to adherens junctions are α-catenin and ZO-1 (reviewed in Refs. [6,7]). The latter protein also recruits ZO-2 into adherens junctions [112]. Cadherins have been shown to bind microtubules directly and thereby may regulate microtubule stability [99]. Moreover, dynein can link microtubules via β-catenin to adherens junctions [100]. Another protein that might link microtubules to adherens junctions via β-catenin is APC [101]. The latter two proteins are important players in the Wnt signalling pathway. Cadherin might sequester β-catenin from the Wnt pathway (reviewed in Ref. [2], see also Section 3.2.2), a function also proposed for ZO-1 [113]. β-Catenin is, like Cx43, a substrate for CK1. Whereas Cx43 phosphorylation by CK1 might play a role in gap junction assembly (Section 3.4.4, Ref. [87]), β-catenin phosphorylation by CK1 is the initial step leading to degradation [114].

Another striking similarity between adherens junctions and gap junctions is the regulation by tyrosine phosphorylation. Active Src has been shown to inhibit cell–cell adhesion, concomitant with tyrosine phosphorylation of N-cadherins and catenins [115]. This can be counteracted by certain protein tyrosine phosphatases [116]. Of note, Src-

mediated tyrosine phosphorylation of the adherens junction component p120^{ctn}, which correlates with decreased adhesion [117], might be counteracted by RPTPμ [118].

The fact that several proteins at the cytoplasmic face of adherens junctions and gap junctions are identical suggests that these subcellular domains are tightly regulated. Moreover, gap junctions might play a similar role as adherens junctions in cytoskeletal anchorage via both actin-ZO-1 and microtubules. Finally, the channel-independent effects of gap junctions might be explained by modulation of transcriptional (co)factors.

4.2. Tight junctions

Tight junctions fuse the lateral margins of contiguous epithelial and endothelial cells together and thereby form a continuous selective barrier between these cells and the extracellular space (Fig. 2). In addition, tight junctions maintain the strict organisation of the plasma membrane of epithelial cells in an apical and a basolateral compartment (Fig. 2). Several growth factors that regulate GJC also regulate tight junction permeability, for instance TNF-α [119]. Some growth factors might close gap junctions and simultaneously increase the tight junction function to isolate cells in stress situations.

The structural integral membrane proteins of tight junctions are the claudins, which have the same overall topology as connexins [120]. Clearly distinguishable in EM, adjacent tight junctions and gap junctions have been found, and the core proteins have been reported to co-immunoprecipitate (see Section 3.2.3). This suggests the co-existence of these junctions at the subcellular level. The zona occludens proteins can cross-linking actin and cortactin to the claudins and occludins (reviewed in Refs. [4,5]). In addition, the ZO-proteins might recruit signalling components, like G-proteins, atypical PKC, ASIP (a PKC binding protein), transport proteins like sec6/8, rab3B/13 and other components found in tight junctions (cingulin, symplexin, 7H6 antigen, VAP33; reviewed in Refs. [4,5]; Fig. 2). ZO-1 can be phosphorylated by kinases acting downstream of the receptors described above, resulting in modulation of tight junction permeability [121].

Interestingly, ZO-1 may be critically involved in regulation of gene expression. Mutant ZO-1 (the PDZ domains) no longer localises to the plasma membrane and can induce an epithelium-to-mesenchymal transition, which is paralleled by activation of the β-catenin pathway. Downregulation of β-catenin signalling by APC overexpression reverts this effect [113]. Moreover, overexpression of β-catenin downregulates ZO-1 levels [122]. Recently, ZO-1 has been shown to sequester and downregulate transcription repressor protein, ZONAB [123] and thus plays a role in transcription regulation. The novel discovered signal transduction properties of tight junctions and adherens junctions have been nicely reviewed [52]. In analogy with the permeability-unrelated effects at tight junctions, with ZO-1 at central stage, gap junctional ZO-1 is a candidate to regulate gene transcription via β-catenin and ZONAB. Furthermore, ZO-1 might serve to link gap junctions to the actin cytoskeleton as has been described for tight junctions and adherens junctions.

5. Concluding remarks and prospective

In the past decade, it has become clear that gap junctions do not consist only of the pore forming connexins (nicely illustrated by Unger et al. [14]), but also of several other proteins.

Kinases, phosphatases and other molecules regulated by receptor stimulation, subcellular localisation or cell cycle are in a delicate balance to dictate the final effect on connexins. Src abrogates GJC by enhancing Cx43 tyrosine phosphorylation resulting in a Cx43/Src complex. Serine and threonine kinases that phosphorylate Cx43 are mainly involved in connexin transport, gap junction assembly, internalisation and degradation. Several serine/threonine kinases, in particular PKCs, have also been implicated in direct modulation of gating, but opposing effects have been found and the signalling mechanisms remain unclear.

Structural proteins recently found at gap junctions, ZO-1 and microtubules, were known to be present at tight junctions and/or adherens junctions. Microtubules interaction might serve to anchor the cytoskeleton to the plasma membrane as has been described for adherens junctions. An additional mechanism of gap junction interaction with the cytoskeleton might be via ZO-1/actin as was found previously in adherens junctions and tight junctions. Several of the major structural proteins of these two junctions (claudin, occludin, cadherin, β-catenin and p120^{ctn}) have been found to co-immunoprecipiate and/or to co-localize with connexins. Future studies, including functional and site-directed mutagenesis, should reveal whether these interactions are direct or reflect precipitation of macromolecular/subcellular complexes.

The channel-independent effects of connexins, which have now been reported by many groups, might be explained by (dys)function of connexin-tail interacting protein(s). Attractive candidates are transcription (co)factors, like ZONAB binding to ZO-1 at tight junctions, or regulators of the Wnt pathway, like β-catenin as postulated for tight junctions. Another attractive candidate that might be downregulated by the Cx43 tail is Src. Future studies should answer if connexins play indeed an important channel-independent role in vivo and reveal the function of the connexin binding partners in (cell) biology.

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Due to space restraints, a number of important research papers could not be included.

References

[1] Garrod DR, Merritt AJ, Nie Z. Desmosomal adhesion: structural basis, molecular mechanism and regulation. Mol Membr Biol 2002;19: 81–94.
[2] Jamora C, Fuchs E. Intercellular adhesion, signalling and the cytoskeleton. Nat Cell Biol 2002;4:E101–8.
[3] Mitic LL, Anderson JM. Molecular architecture of tight junctions. Annu Rev Physiol 1998;60:121–42.
[4] Tsukita S, Furuse M, Itoh M. Structural and signalling molecules come together at tight junctions. Curr Opin Cell Biol 1999;11: 628–33.
[5] Matter K, Balda MS. Signalling to and from tight junctions. Nat Rev, Mol Cell Biol 2003;4:225–36.
[6] Gumbiner BM. Regulation of cadherin adhesive activity. J Cell Biol 2000;148:399–404.
[7] Perez-Moreno M, Jamora C, Fuchs E. Sticky business: orchestrating cellular signals at adherens junctions. Cell 2003;112:535–48.
[8] Willecke K, Eiberger J, Degen J, et al. Structural and functional diversity of connexin genes in the mouse and human genome. Biol Chem 2002;383:725–37.
[9] Bruzzone R, White TW, Paul DL. Connections with connexins: the molecular basis of direct intercellular signaling. Eur J Biochem 1996;238:1–27.
[10] Alexander DB, Goldberg GS. Transfer of biologically important molecules between cells through gap junction channels. Curr Med Chem 2003;10:2045–58.
[11] Goodenough DA, Goliger JA, Paul DL. Connexins, connexons, and intercellular communication. Annu Rev Biochem 1996;65:475–502.
[12] Kumar NM, Gilula NB. The gap junction communication channel. Cell 1996;84:381–8.
[13] Perkins GA, Goodenough DA, Sosinsky GE. Formation of the gap junction intercellular channel requires a 30 degree rotation for interdigitating two apposing connexons. J Mol Biol 1998;277:171–7.
[14] Unger VM, Kumar NM, Gilula NB, Yeager M. Three-dimensional structure of a recombinant gap junction membrane channel. Science 1999;283:1176–80.
[15] Zhou L, Kasperek EM, Nicholson BJ. Dissection of the molecular basis of pp60 (v-src) induced gating of connexin 43 gap junction channels. J Cell Biol 1999;144:1033–45.
[16] Simon AM, Goodenough DA, Paul DL. Mice lacking connexin40 have cardiac conduction abnormalities characteristic of atrioventricular block and bundle branch block. Curr Biol 1998;8:295–8.
[17] Kirchhoff S, Nelles E, Hagendorff A, et al. Reduced cardiac conduction velocity and predisposition to arrhythmias in connexin40-deficient mice. Curr Biol 1998;8:299–302.
[18] Goldberg GS, Lampe PD, Nicholson BJ. Selective transfer of endogenous metabolites through gap junctions composed of different connexins. Nat Cell Biol 1999;1:457–9.
[19] Goldberg GS, Moreno AP, Lampe PD. Gap junctions between cells expressing connexin 43 or 32 show inverse permselectivity to adenosine and ATP. J Biol Chem 2002;277:36725–30.
[20] Saez JC, Connor JA, Spray DC, Bennett MV. 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 1989;86: 2708–12.
[21] Lin JH, Weigel H, Cotrina ML, et al. Gap-junction-mediated propagation and amplification of cell injury. Nat Neurosci 1998;1: 494–500.
[22] Vozzi C, Ullrich S, Charollais A, et al. Adequate connexin-mediated coupling is required for proper insulin production. J Cell Biol 1995; 131:1561–72.
[23] Chen SC, Pelletier DB, Ao P, Boynton AL. Connexin43 reverses the phenotype of transformed cells and alters their expression of cyclin/cyclin-dependent kinases. Cell Growth Differ 1995;6:681–90.
[24] Lo CW, Waldo KL, Kirby ML. Gap junction communication and the modulation of cardiac neural crest cells. Trends Cardiovasc Med 1999;9:63–9.
[25] Mehta PP, Hotz-Wagenblatt A, Rose B, et al. Incorporation of the gene for a cell–cell channel protein into transformed cells leads to normalization of growth. J Membr Biol 1991;124:207–25.
[26] Naus CC. Gap junctions and tumour progression. Can J Physiol Pharmacol 2002;80:136–41.
[27] Atkinson MM, Sheridan JD. Altered junctional permeability between cells transformed by v-ras, v-mos, or v-src. Am J Physiol 1988;255: C674–83.
[28] Omori Y, Yamasaki H. Gap junction proteins connexin32 and connexin43 partially acquire growth-suppressive function in HeLa cells by deletion of their C-terminal tails. Carcinogenesis 1999;20: 1913–8.
[29] Moorby C, Patel M. Dual functions for connexins: Cx43 regulates growth independently of gap junction formation. Exp Cell Res 2001;271:238–48.
[30] Olbina G, Eckhart W. Mutations in the second extracellular region of connexin 43 prevent localization to the plasma membrane, but do not affect its ability to suppress cell growth. Mol Cancer Res 2003;1: 690–700.
[31] Dang X, Doble BW, Kardami E. The carboxy-tail of connexin-43 localizes to the nucleus and inhibits cell growth. Mol Cell Biochem 2003;242:35–8.
[32] Zhang YW, Kaneda M, Morita I. The gap junction-independent tumor suppressing effect of connexin 43. J Biol Chem 2003;278: 44852–6.
[33] Mills SL, Massey SC. Differential properties of two gap junctional pathways made by AII amacrine cells. Nature 1995;377:734–7.
[34] Songyang Z, Fanning AS, Fu C, et al. Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. Science 1997; 275:73–7.
[35] Giepmans BN, Moolenaar WH. The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein. Curr Biol 1998;8:931–4.
[36] Giepmans BN, Hengeveld T, Postma FR, Moolenaar WH. Interaction of c-Src with gap junction protein connexin-43. Role in the regulation of cell–cell communication. J Biol Chem 2001;276: 8544–9.
[37] Toyofuku T, Yabuki M, Otsu K, et al. Direct association of the gap junction protein connexin-43 with ZO-1 in cardiac myocytes. J Biol Chem 1998;273:12725–31.
[38] Kausalya PJ, Reichert M, Hunziker W. Connexin45 directly binds to ZO-1 and localizes to the tight junction region in epithelial MDCK cells. FEBS Lett 2001;505:92–6.
[39] Laing JG, Manley-Markowski RN, Koval M, et al. Connexin45 interacts with zonula occludens-1 and connexin43 in osteoblastic cells. J Biol Chem 2001;276:23051–5.
[40] Olson CO, Li X, Lu S, Nagy JI. Cx36 distribution in the CNS and interaction with Zonula Occludens protein-1 (ZO-1). 2003; No. 476.23. Washington, DC:Soc. Neurosci. Online.
[41] Giepmans BN, Verlaan I, Moolenaar WH. Connexin-43 interactions with ZO-1 and alpha-and beta-tubulin. Cell Commun Adhes 2001;8: 219–23.

[42] Lauf U, Giepmans BN, Lopez P, et al. Dynamic trafficking and delivery of connexons to the plasma membrane and accretion to gap junctions in living cells. Proc Natl Acad Sci U S A 2002;99: 10446–51.
[43] Gaietta G, Deerinck TJ, Adams SR, et al. Multicolor and electron microscopic imaging of connexin trafficking. Science 2002;296: 503–7.
[44] Hunter AW, Jourdan J, Gourdie R. Fusion of GFP to the carboxyl terminus of connexin43 increases gap junction size in HeLa cells. Cell Commun Adhes 2003;10:211–4.
[45] Jongen WM, Fitzgerald DJ, Asamoto M, et al. Regulation of connexin 43-mediated gap junctional intercellular communication by Ca^{2+} in mouse epidermal cells is controlled by E-cadherin. J Cell Biol 1991;114:545–55.
[46] Meyer RA, Laird DW, Revel JP, Johnson RG. Inhibition of gap junction and adherens junction assembly by connexin and A-CAM antibodies. J Cell Biol 1992;119:179–89.
[47] Fujimoto K, Nagafuchi A, Tsukita S, et al. Dynamics of connexins, E-cadherin and alpha-catenin on cell membranes during gap junction formation. J Cell Sci 1997;110:311–22.
[48] Hertig CM, Butz S, Koch S, et al. N-Cadherin in adult rat cardiomyocytes in culture: II. Spatio-temporal appearance of proteins involved in cell–cell contact and communication. Formation of two distinct N-cadherin/catenin complexes. J Cell Sci 1996;109:11–20.
[49] Ai Z, Fischer A, Spray DC, et al. Wnt-1 regulation of connexin43 in cardiac myocytes. J Clin Invest 2000;105:161–71.
[50] Xu X, Li WE, Huang GY, et al. Modulation of mouse neural crest cell motility by N-cadherin and connexin 43 gap junctions. J Cell Biol 2001;154:217–30.
[51] Heasman J, Crawford A, Goldstone K, et al. Overexpression of cadherins and underexpression of beta-catenin inhibit dorsal mesoderm induction in early Xenopus embryos. Cell 1994;79: 791–803.
[52] Balda MS, Matter K. Epithelial cell adhesion and the regulation of gene expression. Trends Cell Biol 2003;13:310–8.
[53] Kojima T, Sawada N, Chiba H, et al. Induction of tight junctions in human connexin 32 (hCx32)-transfected mouse hepatocytes: connexin 32 interacts with occludin. Biochem Biophys Res Commun 1999;266:222–9.
[54] Kojima T, Kokai Y, Chiba H, et al. Cx32 but not Cx26 is associated with tight junctions in primary cultures of rat hepatocytes. Exp Cell Res 2001;263:193–201.
[55] Loewenstein WR. Junctional intercellular communication and the control of growth. Biochim Biophys Acta 1979;560:1–65.
[56] Crow DS, Beyer EC, Paul DL, et al. Phosphorylation of connexin43 gap junction protein in uninfected and Rous sarcoma virus-transformed mammalian fibroblasts. Mol Cell Biol 1990;10: 1754–63.
[57] Swenson KI, Piwnica-Worms H, McNamee H, Paul DL. Tyrosine phosphorylation of the gap junction protein connexin43 is required for the pp60v-src-induced inhibition of communication. Cell Regul 1990;1:989–1002.
[58] Kanemitsu MY, Loo LW, Simon S, et al. Tyrosine phosphorylation of connexin 43 by v-Src is mediated by SH2 and SH3 domain interactions. J Biol Chem 1997;272:22824–31.
[59] Lin R, Warn-Cramer BJ, Kurata WE, Lau AF. v-Src phosphorylation of connexin 43 on Tyr247 and Tyr265 disrupts gap junctional communication. J Cell Biol 2001;154:815–27.
[60] Hii CS, Oh SY, Schmidt SA, et al. Lysophosphatidic acid inhibits gap-junctional communication and stimulates phosphorylation of connexin-43 in WB cells: possible involvement of the mitogen-activated protein kinase cascade. Biochem J 1994;303:475–9.
[61] Postma FR, Hengeveld T, Alblas J, et al. Acute loss of cell–cell communication caused by G protein-coupled receptors: a critical role for c-Src. J Cell Biol 1998;140:1199–209.
[62] Spinella F, Rosano L, DiCastro V, et al. Endothelin-1 decreases gap-junctional intercellular communication by inducing phosphorylation of connexin 43 in human ovarian carcinoma cells. J Biol Chem 2003;278:41294–301.
[63] Huang S, Dudez T, Scerri I, et al. Defective activation of c-Src in cystic fibrosis airway epithelial cells results in loss of tumor necrosis factor-alpha-induced gap junction regulation. J Biol Chem 2003;278: 8326–32.
[64] Lidington D, Tyml K, Ouellette Y. Lipopolysaccharide-induced reductions in cellular coupling correlate with tyrosine phosphorylation of connexin 43. J Cell Physiol 2002;193:373–9.
[65] Toyofuku T, Yabuki M, Otsu K, et al. Functional role of c-Src in gap junctions of the cardiomyopathic heart. Circ Res 1999;85:672–81.
[66] Toyofuku T, Zhang H, Akamatsu Y, et al. c-Src regulates the interaction between connexin-43 and ZO-1 in cardiac myocytes. J Biol Chem 2001;276:1780–8.
[67] Mikalsen SO, Kaalhus O. A characterization of pervanadate, an inducer of cellular tyrosine phosphorylation and inhibitor of gap junctional intercellular communication. Biochim Biophys Acta 1996;1290:308–18.
[68] Zhang YW, Morita I, Nishida M, Murota SI. Involvement of tyrosine kinase in the hypoxia/reoxygenation-induced gap junctional intercellular communication abnormality in cultured human umbilical vein endothelial cells. J Cell Physiol 1999;180:305–13.
[69] van Veen TA, van Rijen HV, Jongsma HJ. Electrical conductance of mouse connexin45 gap junction channels is modulated by phosphorylation. Cardiovasc Res 2000;46:496–510.
[70] Giepmans BN, Feiken E, Gebbink MF, Moolenaar WH. Association of connexin43 with a receptor protein tyrosine phosphatase. Cell Commun Adhes 2003;10:201–5.
[71] Lampe PD, Tenbroek EM, Burt JM, et al. Phosphorylation of connexin43 on serine368 by protein kinase C regulates gap junctional communication. J Cell Biol 2000;149:1503–12.
[72] Doble BW, Ping P, Kardami E. The epsilon subtype of protein kinase C is required for cardiomyocyte connexin-43 phosphorylation. Circ Res 2000;86:293–301.
[73] Lin D, Boyle DL, Takemoto DJ. IGF-I-induced phosphorylation of connexin 43 by PKCgamma: regulation of gap junctions in rabbit lens epithelial cells. Invest Ophthalmol Vis Sci 2003;44:1160–8.
[74] Weng S, Lauven M, Schaefer T, et al. Pharmacological modification of gap junction coupling by an antiarrhythmic peptide via protein kinase C activation. FASEB J 2002;16:1114–6.
[75] Tenbroek EM, Lampe PD, Solan JL, et al. Ser364 of connexin43 and the upregulation of gap junction assembly by cAMP. J Cell Biol 2001;155:1307–18.
[76] Yogo K, Ogawa T, Akiyama M, et al. Identification and functional analysis of novel phosphorylation sites in Cx43 in rat primary granulosa cells. FEBS Lett 2002;531:132–6.
[77] Kwak BR, Saez JC, Wilders R, et al. Effects of cGMP-dependent phosphorylation on rat and human connexin43 gap junction channels. Pflugers Arch 1995;430:770–8.
[78] Chiba H, Sawada N, Oyamada M, et al. Hormonal regulation of connexin 43 expression and gap junctional communication in human osteoblastic cells. Cell Struct Funct 1994;19:173–7.
[79] Kanemitsu MY, Lau AF. Epidermal growth factor stimulates the disruption of gap junctional communication and connexin43 phosphorylation independent of 12-*O*-tetradecanoylphorbol 13-acetate-sensitive protein kinase C: the possible involvement of mitogen-activated protein kinase. Mol Biol Cell 1993;4:837–48.
[80] Pelletier DB, Boynton AL. Dissociation of PDGF receptor tyrosine kinase activity from PDGF-mediated inhibition of gap junctional communication. J Cell Physiol 1994;158:427–34.
[81] Hossain MZ, Jagdale AB, Ao P, et al. Disruption of gap junctional communication by the platelet-derived growth factor is mediated via multiple signaling pathways. J Biol Chem 1999;274:10489–96.
[82] Vikhamar G, Rivedal E, Mollerup S, Sanner T. Role of Cx43 phosphorylation and MAP kinase activation in EGF induced enhancement of cell communication in human kidney epithelial cells. Cell Commun Adhes 1998;5:451–60.

[83] Le AC, Musil LS. A novel role for FGF and extracellular signal-regulated kinase in gap junction-mediated intercellular communication in the lens. J Cell Biol 2001;154:197–216.
[84] Polontchouk L, Ebelt B, Jackels M, Dhein S. Chronic effects of endothelin 1 and angiotensin II on gap junctions and intercellular communication in cardiac cells. FASEB J 2002;16:87–9.
[85] Lampe PD, Kurata WE, Warn-Cramer BJ, Lau AF. Formation of a distinct connexin43 phosphoisoform in mitotic cells is dependent upon p34cdc2 kinase. J Cell Sci 1998;111:833–41.
[86] Kanemitsu MY, Jiang W, Eckhart W. Cdc2-mediated phosphorylation of the gap junction protein, connexin43, during mitosis. Cell Growth Differ 1998;9:13–21.
[87] Cooper CD, Lampe PD. Casein kinase 1 regulates connexin-43 gap junction assembly. J Biol Chem 2002;277:44962–8.
[88] Cheng HL, Louis CF. Endogenous casein kinase I catalyzes the phosphorylation of the lens fiber cell connexin49. Eur J Biochem 1999;263:276–86.
[89] Cheng HL, Louis CF. Functional effects of casein kinase I-catalyzed phosphorylation on lens cell-to-cell coupling. J Membr Biol 2001; 181:21–30.
[90] Yin X, Jedrzejewski PT, Jiang JX. Casein kinase II phosphorylates lens connexin 45.6 and is involved in its degradation. J Biol Chem 2000;275:6850–6.
[91] Lampe PD, Lau AF. The effects of connexin phosphorylation on gap junctional communication. Int J Cancer 2003 [in press].
[92] Musil LS, Goodenough DA. Biochemical analysis of connexin43 intracellular transport, phosphorylation, and assembly into gap junctional plaques. J Cell Biol 1991;115:1357–74.
[93] Falk MM. Cell-free synthesis for analyzing the membrane integration, oligomerization, and assembly characteristics of gap junction connexins. Methods 2000;20:165–79.
[94] Beardslee MA, Laing JG, Beyer EC, Saffitz JE. Rapid turnover of connexin43 in the adult rat heart. Circ Res 1998;83:629–35.
[95] Guan X, Ruch RJ. Gap junction endocytosis and lysosomal degradation of connexin43-P2 in WB-F344 rat liver epithelial cells treated with DDT and lindane. Carcinogenesis 1996;17:1791–8.
[96] Thomas MA, Huang S, Cokoja A, et al. Interaction of connexins with protein partners in the control of channel turnover and gating. Biol Cell 2002;94:445–56.
[97] Giepmans BN, Verlaan I, Hengeveld T, et al. Gap junction protein connexin-43 interacts directly with microtubules. Curr Biol 2001;11: 1364–8.
[98] Waterman-Storer CM, Salmon WC, Salmon ED. Feedback interactions between cell–cell adherens junctions and cytoskeletal dynamics in newt lung epithelial cells. Mol Biol Cell 2000;11: 2471–83.
[99] Chausovsky A, Bershadsky AD, Borisy GG. Cadherin-mediated regulation of microtubule dynamics. Nat Cell Biol 2000;2:797–804.
[100] Ligon LA, Karki S, Tokito M, Holzbaur EL. Dynein binds to beta-catenin and may tether microtubules at adherens junctions. Nat Cell Biol 2001;3:913–7.
[101] Zumbrunn J, Kinoshita K, Hyman AA, Nathke IS. Binding of the adenomatous polyposis coli protein to microtubules increases microtubule stability and is regulated by GSK3 phosphorylation. Curr Biol 2001;11:44–9.
[102] Itoh M, Nagafuchi A, Moroi S, Tsukita S. Involvement of ZO-1 in cadherin-based cell adhesion through its direct binding to alpha catenin and actin filaments. J Cell Biol 1997;138:181–92.
[103] Itoh M, Yonemura S, Nagafuchi A, et al. A 220-kD undercoat-constitutive protein: its specific localization at cadherin-based cell–cell adhesion sites. J Cell Biol 1991;115:1449–62.
[104] Schubert AL, Schubert W, Spray DC, Lisanti MP. Connexin family members target to lipid raft domains and interact with caveolin-1. Biochemistry 2002;41:5754–64.
[105] Welsh MJ, Aster JC, Ireland M, et al. Calmodulin binds to chick lens gap junction protein in a calcium-independent manner. Science 1982;216:642–4.
[106] Peracchia C, Sotkis A, Wang XG, et al. Calmodulin directly gates gap junction channels. J Biol Chem 2000;275:26220–4.
[107] Brigstock DR. The CCN family: a new stimulus package. J Endocrinol 2003;178:169–75.
[108] McLeod TL, Bechberger JF, Naus CC. Determination of a potential role of the CCN family of growth regulators in connexin43 transfected C6 glioma cells. Cell Commun Adhes 2001;8:441–5.
[109] Singer KL, Stevenson BR, Woo PL, Firestone GL. Relationship of serine/threonine phosphorylation/dephosphorylation signaling to glucocorticoid regulation of tight junction permeability and ZO-1 distribution in nontransformed mammary epithelial cells. J Biol Chem 1994;269:16108–15.
[110] Willott E, Balda MS, Fanning AS, et al. The tight junction protein ZO-1 is homologous to the Drosophila discs-large tumor suppressor protein of septate junctions. Proc Natl Acad Sci U S A 1993;90:7834–8.
[111] Itoh M, Nagafuchi A, Yonemura S, et al. The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. J Cell Biol 1993;121: 491–502.
[112] Itoh M, Morita K, Tsukita S. Characterization of ZO-2 as a MAGUK family member associated with tight as well as adherens junctions with a binding affinity to occludin and alpha catenin. J Biol Chem 1999;274:5981–6.
[113] Reichert M, Muller T, Hunziker W. The PDZ domains of zonula occludens-1 induce an epithelial to mesenchymal transition of Madin–Darby canine kidney I cells. Evidence for a role of beta-catenin/tcf/lef signaling. J Biol Chem 2000;275:9492–500.
[114] Polakis P. Casein kinase 1: a Wnt'er of disconnect. Curr Biol 2002;12: R499–501.
[115] Hamaguchi M, Matsuyoshi N, Ohnishi Y, et al. p60v-src causes tyrosine phosphorylation and inactivation of the N-cadherin–catenin cell adhesion system. EMBO J 1993;12:307–14.
[116] Taddei ML, Chiarugi P, Cirri P, et al. Beta-catenin interacts with low-molecular-weight protein tyrosine phosphatase leading to cadherin-mediated cell–cell adhesion increase. Cancer Res 2002;62:6489–99.
[117] Ozawa M, Ohkubo T. Tyrosine phosphorylation of p120(ctn) in v-Src transfected L cells depends on its association with E-cadherin and reduces adhesion activity. J Cell Sci 2001;114:503–12.
[118] Zondag GC, Reynolds AB, Moolenaar WH. Receptor protein-tyrosine phosphatase RPTPmu binds to and dephosphorylates the catenin p120(ctn). J Biol Chem 2000;275:11264–9.
[119] Schmitz H, Fromm M, Bentzel CJ, et al. Tumor necrosis factor-alpha (TNFalpha) regulates the epithelial barrier in the human intestinal cell line HT-29/B6. J Cell Sci 1999;112:137–46.
[120] Morita K, Furuse M, Fujimoto K, Tsukita S. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. Proc Natl Acad Sci U S A 1999;96: 511–6.
[121] Van Itallie CM, Balda MS, Anderson JM. Epidermal growth factor induces tyrosine phosphorylation and reorganization of the tight junction protein ZO-1 in A431 cells. J Cell Sci 1995;108:1735–42.
[122] Mann B, Gelos M, Siedow A, et al. Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. Proc Natl Acad Sci U S A 1999;96:1603–8.
[123] Balda MS, Matter K. The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. EMBO J 2000;19: 2024–33.
[124] Pawson T, Scott JD. Signaling through scaffold, anchoring, and adaptor proteins. Science 1997;278:2075–80.
[125] Barker RJ, Price RL, Gourdie RG. Increased association of ZO-1 with connexin43 during remodeling of cardiac gap junctions. Circ Res 2002;90:317–24.
[126] Saez JC, Nairn AC, Czernik AJ, et al. Phosphorylation of connexin43 and the regulation of neonatal rat cardiac myocyte gap junctions. J Mol Cell Cardiol 1997;29:2131–45.
[127] Warn-Cramer BJ, Lampe PD, Kurata WE, et al. Characterization of

the mitogen-activated protein kinase phosphorylation sites on the connexin-43 gap junction protein. J Biol Chem 1996;271:3779–86.
[128] Thomas MA, Zosso N, Scerri I, et al. A tyrosine-based sorting signal is involved in connexin43 stability and gap junction turnover. J Cell Sci 2003;116:2213–22.
[129] Nielsen PA, Baruch A, Shestopalov VI, et al. Lens connexins alpha3Cx46 and alpha8Cx50 interact with zonula occludens protein-1 (ZO-1). Mol Biol Cell 2003;14:2470–81.
[130] Nielsen PA, Beahm DL, Giepmans BN, et al. Molecular cloning, functional expression, and tissue distribution of a novel human gap junction-forming protein, connexin-31.9. Interaction with zona occludens protein-1. J Biol Chem 2002;277:38272–83.