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Review

# Gap junction proteins on the move: Connexins, the cytoskeleton and migration ☆

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ABSTRACT

Connexin43 (Cx43) has roles in cell–cell communication as well as channel independent roles in regulating motility and migration. Loss of function approaches to decrease Cx43 protein levels in neural cells result in reduced migration of neurons during cortical development in mice and impaired glioma tumor cell migration. In other cell types, correlations between Cx43 expression and cell morphology, adhesion, motility and migration have been noted. In this review we will discuss the common themes that have been revealed by a detailed comparison of the published results of neuronal cells with that of other cell types. In brief, these comparisons clearly show differences in the stability and directionality of protrusions, polarity of movement, and migration, depending on whether a) residual Cx43 levels remain after siRNA or shRNA knockdown, b) Cx43 protein levels are not detectable as in cells from Cx43 $^{-/-}$ knockout mice or in cells that normally have no endogenous Cx43 expression, c) gain-of-function approaches are used to express Cx43 in cells that have no endogenous Cx43 and, d) Cx43 is over-expressed in cells that already have low endogenous Cx43 protein levels. What is clear from our comparisons is that Cx43 expression influences the adhesiveness of cells and the directionality of cellular processes. These observations are discussed in light of the ability of cells to rearrange their cytoskeleton and move in an organized manner. This article is part of a Special Issue entitled: The Communicating junctions, roles and dysfunctions.



**Contents**



## 1. Introduction

Gap junctions (GJs) have long been considered important for their role as membrane channels, but it is now clear that the proteins which form these channels, the connexins (Cxs), are multi-modal in their structure and function. Thus along with their function in cell–cell communication, Cxs also have channel independent roles in regulating cell morphology, establishing polarity, and rearrangement of the cytoskeleton, thereby influencing cell movement. The most prominent examples come from

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studies using neural cells. For example, glioma and neuronal cell motility and migration are affected by Cx43 expression [1–4]. Genetic knockout [5], as well as knockdown of conenxin43 (Cx43) protein levels using shRNA constructs [3,4] or by using neuronal progenitor specific conditional knockout mice [2,6], results in reduced migration of neurons in the developing cortex. There is an abundance of evidence in the literature that describes effects of Cxs and GJs on cell adhesion, motility and migration. In this review we will discuss the common themes that are revealed by a detailed comparison of the results using neural cells with that of other cell types. For the purpose of this review, we have focused on Cx43 since this connexin, one of the first to be identified, is most widely and abundantly expressed, and the unique long carboxyl-terminal tail with multiple protein interaction sites provides interesting opportunities for its participation in intracellular interactions and signaling, and thus potential impact on cytoskeletal events associated with changes in cell shape, cell polarization and migration. However it should be noted that other connexins, some of which are co-expressed with Cx43, could impact cell migration, depending on the cell or tissue system.

What becomes clear from these comparisons is that Cx43 expression can influence the effectiveness of forward cell movement, due to the directionality of cellular protrusions. Cells initiate movement through their ability to breakdown and reform the actin cytoskeleton network, and to stabilize it, as cell protrusions develop into leading edges that support forward movement. We will address these issues as they relate to Cx43 expression and discuss the approaches used. The directionality of protrusions, polarity of movement, and effectiveness of migration, depends on whether a) residual Cx43 levels remain after siRNA or shRNA knockdown, b) Cx43 protein levels are not detectable as in cells from $Cx43^{-/-}$ knockout mice or in cells that normally have no endogenous Cx43 expression (i.e. plasmacytoma/myeloma tumor cells), c) gain-of-function approaches are used to express Cx43 in cells that have no endogenous Cx43 and, d) Cx43 is over-expressed in cells that already have low endogenous Cx43 protein levels. Table 1 summarizes a number of studies using different cell systems that fall into each of these categories. What is clear from these comparisons is that Cx43 expression influences the adhesiveness of cells and the directionality of cellular process extensions. These two characteristics profoundly influence the ability of cells to rearrange their cytoskeleton and move in an organized manner, in a productive forward direction.

## 2. Overview of gap junctions

The gap junction (GJ) protein Cx43 is expressed in many cell types including fibroblasts, epithelial cells, hematopoietic cells, neurons, and cardiac neuronal crest cells [7–9]. Cx43 monomers combine to form hexameric hemichannels called connexons that can associate with connexons on adjacent cells to form GJs, allowing for the intercellular passage of ions and small molecules (generally less than 1 kDa) such as $Ca^{++}$, ATP and cAMP (reviewed in [7]). Highly organized GJ plaques appear to be restricted between adjacent adherent cells in organized cell layers. However, recent evidence has also shown that connexons can function as hemichannels for specific purposes in some cell types [10]. Other GJ family members, namely the pannexins, have been proposed to underlie hemichannel functions [11], and to foster interesting cellular events involving antigen presentation and immune responses [12,13], as well as having tumor suppressive effects [14,15].

## 3. Gap junction structure and function

Intercellular communication is an important process that contributes to developmental events, tissue homeostasis and the establishment and maintenance of niches for different cell types. The passage of cytosolic molecules through channels between cells, or of extracellular molecules from the environment into cells, is mediated by the GJ family of proteins [16]. GJ proteins are members of the tetraspanin integral membrane protein family that when assembled into multimers form channels spanning the plasma membrane. There are three families of GJ-forming proteins: Innexins, which are the invertebrate equivalents of GJ proteins; Connexins, which are chordate GJ proteins; and the more recently identified chordate Pannexins, the vertebrate analog of the Innexins [17,18]. Although these three GJ protein families do not necessarily share sequence homology with each other, they share similarity in their protein structure and membrane topology. Both have cytoplasmic N- and C-terminal domains and the protein spans the membrane four times, with two extracellular loops containing cysteine residues required for proper connexon docking. It is believed that all three groups of proteins assemble into multimers that can also form functional hemichannels as well as classical GJ channels between two adjacent cells, although there is still discussion about the functionality of hemichannels in some cell types. However pannexins preferentially form channels that do not associate into GJ between cells (reviewed in [19]).

There are 20–21 different connexins expressed in vertebrates, depending on the species. One of the main differences among Cx members is in the lengths of the cytosolic loop and C-terminal domain. Fig. 1 summarizes the structural and functional aspects attributed to various regions of Cx43. Cxs are required for electrical coupling of cells, for example, in the co-ordination of contraction of cardiac cells and insulin release from pancreatic beta cells [20,21]. Cxs can also form hemichannels, although this is a controversial topic. The main concern focuses on the presence of pannexin channels which could account for the observed hemichannel effects. This has been extensively covered in other contributions to this review series [22–24]. There are reports that hemichannels can transport ATP and that they can assist in calcium signaling [18,25,26] (and reviewed in [27]). Interestingly, a new role for Cxs in neuronal development and migration that does not involve channel activity has been identified [2–4]. These findings have led to the re-examination of published results using other cell types and this non-channel role for Cxs will be discussed in great detail in this review.

The GJ protein Cx43 is the most well studied of the Cxs due to its widespread expression in tissues [28] and it is the most predominant Cx expressed in most tissue culture cell lines including lymphocytes [7,29]. Genetic knockout of Cx43 results in mice that die shortly after birth due to heart malformation, making study in mouse models dependent on conditional knockouts or in-utero electroporation techniques [2,3,30].

The regulation of the Cx43 channel is partially achieved by phosphorylation and de-phosphorylation of the C-terminal domain by protein kinase C (PKC), Map kinases (MAPK) and the Src family tyrosine kinases [21,31–36]. PKC has been shown to phosphorylate Cx43 on serines 262 and 368, which can lead to a reduction in channel conductivity [21,33]. MAPK can phosphorylate Cx43 on serines 255, 279 and 282 resulting in the downregulation of channel conductivity due to a reduction in the frequency in which the channel is open [34,35]. The phosphorylation of tyrosines 268 and 247 by the Src protein tyrosine kinase is also important for channel regulation. The Src kinases are proposed to be recruited to the proline-rich domain of the C-terminal tail of Cx43 and to bind via its SH3 domain, so that it can then phosphorylate tyrosine 268. This phosphorylation provides a potential SH2 binding site which recruits tyrosine kinases to phosphorylate tyrosine 247, resulting in channel closure [36]. Cx43 also recruits adaptor proteins which link to the cytoskeleton [31]. The tight junctional protein Zonula occludens (ZO)-1 is reported as interacting with the very C-terminus of Cx43 [37] where it is proposed to stabilize and regulate GJ plaque size [38]. ZO-1 also regulates the transition of undocked connexons into GJ aggregates (plaques), thereby regulating the number of hemichannels compared with the number of classical GJ channels at the plasma membrane [39]. The C-terminal domain of Cx43 also interacts with the adaptor protein

**Table 1**
Summary of the effects of Cx43 on cell adhesion, process extension and motility reported in various studies.

| | Cx type | Experimental approach | Adhesion | Process extension | Motility | References |
|---|---|---|---|---|---|---|
| *Overexpression or expression* | | | | | | |
| C6 glioma | Cx43 | Overexpression | ND | Multiple extensions and more flattened | Enhances in wound healing assays and in transwell migration assays; less of an effect with Cx43ΔCT | [1]; Naus et al., unpublished |
| C6 glioma | Cx43 | Overexpression | Increased cell:cell adhesion | ND | ND | [67,138] |
| HeLa | Cx43 | Normally non-expressing; stable transfection | ND | ND | Enhances | [139] |
| GL15, 8-MG, human biopsies, C6 glioma | Cx43 | Endogenous expression; stable transfection | ND | ND | Enhances | [140] |
| C6 glioma | Cx43 | Overexpression; stable transfection | ND | ND | Enhances | [141] |
| J558 μm3 myeloma | none | Normally non-expressing | Normally non-adherent plasma cells (lymphoid) | Small protrusions in response to BCR signaling; No spreading in any direction | ND | [96] |
| J558 μm3 myeloma | Cx43 | Transient transfection of cell populations; sorting expressors by FACS | Increased adhesion to endothelial cells | BCR signaling-induced radial spreading; increased Rap1 GTPase activation | ND | [96] |
| WEHI 231 B-lymphoma | Cx43 | Transient overexpression by transfection of cell populations; sorting high expressors by FACS | ND | Enhanced BCR signaling-mediated spreading | ND | [96] |
| 5TGM1 myeloma | none | Normally non-expressing | Normally non-adherent plasma cells (lymphoid) | ND | ND | [96] |
| 5TGM1 myeloma | Cx43 | Transient transfection of populations | Increased adhesion to endothelial cells | | | [96] |
| *Knockdown* | | | | | | |
| NIH3T3 fibroblasts | Cx43 | Cx43siRNA | ND | ND | Reduces | [44] |
| MDA-MB-231, Hs578T | Cx43 | Cx43siRNA | ND | ND | Reduces | [142] |
| C6 glioma | Cx43 | Cx43shRNA | ND | ND | Reduces | [1] |
| Developing rat brain | Cx43, Cx26 | Cx43shRNA | ND | ND | Reduces | [3] |
| Developing mouse brain | Cx43 | Cx43antisense | ND | ND | Reduces | [2] |
| Skin/epidermis | Cx43 | Cx43siRNA | ND | ND | Reduces | [143] |
| MCF-10A breast epithelial cancer cells | Cx43 | siRNA knockdown screen of 'Migration & Adhesion Related (MAR)' genes, kinases and phosphatases | Disrupted, lose integrity of monolayer; N-cadherin levels down | Lack of polarity of process extension, disorganized, multidirectional orientation | Disorganized migration, erratic movement in different directions; enhanced, accelerated movement | [43] |
| WEHI 231 B-lymphoma | Cx43 | Cx43shRNA | ND | Inhibits BCR mediated radial spreading; decreases Rap1 GTPase activation | Reduces integrin and chemokine mediated motility and migration; decreases Rap1 GTPase activation | [96] [97] |
| A20 B-lymphoma | Cx43 | Cx43shRNA | ND | Inhibits; spreading is not radial, multidirectional processes | ND | Machtaler, Matsuuchi, et al., unpublished |
| Skin lesions | Cx43 | Cx43antisense | ND | ND | Enhances wound closure | [143] |
| *Knockout (KO)* | | | | | | |
| Developing brain | Cx43 | Cx43 KO | ND | ND | Reduces radial migration | [5] [2] |
| Developing brain | Cx43 | Mouse Nestin-Cre; Cx43fl/fl | ND | ND | Reduces radial migration | [2] |
| Neural tube explant | Cx43 | Mouse Cx43 KO | ND | ND | Reduces migration | [52] |
| | Cx43 | Mouse CMV43 | ND | ND | Increases migration | [52] |
| Neurospheres | Cx43 | Culture assay from Cx43 KO mice | ND | ND | Reduces; migration restored by P2Y1 receptor expression | [82] |
| Mouse embryonic fibroblasts | Cx43 | From Cx43 KO mice | ND | More protrusions in different directions; lack of cell polarity; lack of MTOC to orient properly | Wound closure impaired | [50] |

**Table 1** (*continued*)

| | Cx type | Experimental approach | Adhesion | Process extension | Motility | References |
|---|---|---|---|---|---|---|
| Cardiac neural crest cells (CNC) | Cx43 | From Cx43 knockout mice | Some focal adhesions absent or miss-localized | Protrusions/processes extending and retracting in multiple directions; actin stress fibers disorganized, polygonal and not parallel | Impaired forward movement; lack of polarity; process extension is multidirectional | [49] [45] |
| Cardiac neural crest cells explant of spheroid | N-cadherin | From N-cadherin KO mice compared to Cx43 KO mice | ND | ND | Speed of CNC cells was elevated in N-cadherin deficient CNCs, accompanied by a lack of uniform directionality | [45] |
| *Expression of mutants* | | | | | | |
| Developing rat brain | Cx43 Cx26 | Cx shRNA + wild type Cx | ND | ND | Rescue of radial migration | [3] |
| | | Cx shRNA + Cx channel dead | ND | ND | Rescue of radial migration | [3] |
| | | Cx shRNA + Cx Cys mutant | ND | ND | No migration rescue | [3] |
| | | Cx shRNA + Cx43 cytoplasmic tail deletion ( CT) | ND | ND | Rescue of radial migration | [3] |
| | Cx43 Cx26 | Brain slice; Cx shRNA + wild type Cx | ND | ND | No effect on tangential migration, but rescue of switch from tangential to radial migration | [4] |
| Developing mouse brain | Cx43 | Cx43 cytoplasmic tail deletion (ΔCT) | ND | ND | No rescue of radial migration | [2] |
| Developing mouse brain | Cx43 | Expression of Cx43 cytoplasmic tail deletion mutant in nestin-Cre; Cx43fl/fl mice | ND | ND | No rescue of migration | [2] |
| J558μm3 myeloma | Cx43 | Cx43 cytoplasmic tail deletion (ΔCT) | ND | BCR-mediated spreading attenuated; dynamic unstable protrusions seen extending and retracting; Rap1 GTPase activation is impaired | ND | [96] |
| Mouse embryo fibroblasts | Cx43 | From KO mice and mice reconstituted with a Cx43 cytoplasmic tail deletion mutant | ND | Cells showed multiple protrusions that were not polarized | Migration impaired | [50] |
| *Other* | | | | | | |
| Spontaneous canine astrocytoma | Cx43 | EGF stimulation | ND | ND | Attenuates | [144] |
| Skin/epidermis | | Cx43 localization using antibody staining | ND | ND | Attenuates | [145] |
| Proepicardial explants | | Mouse models; Cx43 KO mice | ND | ND | Attenuates | [146] |
| 3T3 A31 fibroblasts | Cx43-256 M | Stable transfection | ND | ND | Mutation attenuates | [147] |

drebrin and co-localizes with cortactin [40,41]. Drebrin is a brain-restricted actin binding protein that belongs to the drebrin/actin binding protein (abp) family [42]. Drebrin was shown, using a combination of co-immunoprecipitation and FRET-based analysis, to interact with the C-terminal domain of Cx43 where it is thought to stabilize the formation of GJs by linking them to the F-actin cytoskeleton [40]. Cortactin, another actin-binding protein, was also shown to interact with Cx43 using a combination of co-immunoprecipitation and immunofluorescence co-localization studies, though its function is not known [41].

## 4. Gap junctions in migration in specific systems

Along with its role in cell–cell communication, there are emerging functions for Cx43 as a regulator of cell morphology, polarity, process extension, adhesiveness, motility and directed migration toward a chemoattractant [1–4]. All of these cellular processes rely on progressive rearrangements of the cytoskeletal network. While it is attractive to propose that these additional functions are due solely to Cx43's channel function, mounting evidence suggests alternative roles of Cx43 as a signaling scaffold or as an adaptor protein to promote the nucleation of cytoskeleton-associated proteins that regulate the disassembly, re-assembly and branching of the actin cytoskeleton or influence the behavior of microtubules. In this part of the review we will discuss the proposed non-channel functions of Cx43 in specific cellular responses in different cell types.

### 4.1. Wound healing breast epithelial cells as a non-biased model

The Cx43 gene (GJA1) was identified by Brugge and colleagues, using a high-throughput siRNA knockdown screen of breast MCF-10A epithelial cells, as having a pivotal role in cell migration [43]. This screen was not specifically designed to examine GJ proteins but instead was focused on identifying kinases and phosphatases important for cell migration. The authors used three specialized gene libraries that

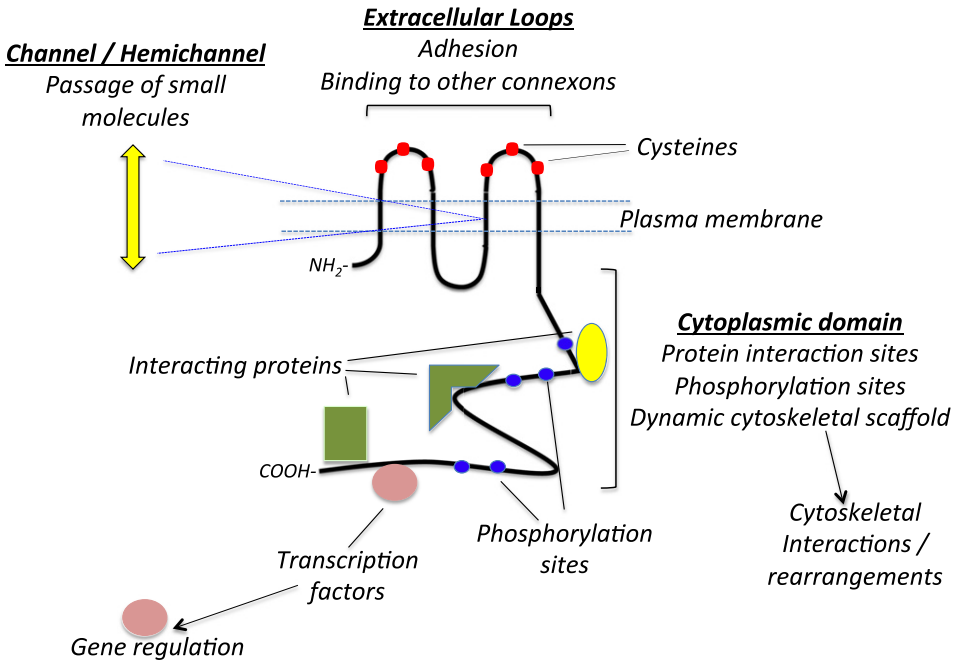


**Fig. 1.** Schematic diagram of Cx43 indicating its membrane topology. The cysteines (red dots) in the extracellular loops are involved in adhesion and in the binding to other connexins on adjacent cells to form gap junctions. Residues in the transmembrane domains of the connexins that are assembled into hexameric connexons, line the hydrophilic channel and facilitate the passage of small molecules through the gap junction pore. The cytoplasmic tail of Cx43 contains multiple protein interaction sites (proteins shown as yellow and green shapes), as well as serine, threonine and tyrosine residues (blue dots) that can be phosphorylated. These interactions and modifications regulate Cx43 function.

contained a) kinases (576 genes for protein or lipid kinase genes), b) phosphatases (192 genes) and as a control, c) the Migration and Adhesion Related (MAR) collection of 313 genes from Dr. Benny Geiger (Weitzmann Institute, Israel). A significant knockdown of protein (in the range of 70% of wild type levels) was achieved by introducing into cells the vast majority of the siRNAs used in this screen. The effect on cell migration was assayed using time-lapse imaging of a wound-healing assay with MCF-10A breast epithelial cells. Individual cell pathways were tracked over a 12-hour period revealing previously unseen diversions in each cell's direction. Confirmation of these effects on migration of the most pronounced genes was obtained using a second cell line and the results, including videos of the time-lapse images, are available at a fully interactive database hosted by the Cell Migration Consortium (www.cellmigration.org/pubs/wound_rnai.htm).

Please see information page for the GJA1 gene (www.cellmigration.org/resource/discovery/brugge/simpson2008_rnai.cgi?my_table=table&lookup_text=sy_descr&symbol=GJA1&submit=Search&cat_count=1&lookup_cat=none&cat_values=0&lookup_cat1=none&cat_values1=0&sort=sy&limit=20&order=0&begin=0&end=20).

The Cx43 gene (GJA1) was classified as one of 13 'accelerated migration' Class C genes and was discussed in the main body of the paper. When GJA1 was knocked down the cells filled in the wound faster, but it was noted that migration of the cells was aberrant, less directed, erratic, with cells showing poorly defined or non-existent front and rear end polarity. The time-lapse images captured every 6 min over 20 h of 8 individual cells tracked the dynamic nature of the wound edge. While wild-type cells traveled uniformly in the same direction from the wound edge, with little deviation, the GJA1 knockdown cells' pathways were erratic, going in different directions, reversing direction and were not uniform. They described the knockdown cells as showing significant alterations in cell–cell adhesion, impaired adhesion to the substrate, multiple changes in directionality of movement, alterations in cell polarity, and changes in leading edge morphology and dynamics. Cell protrusions were not uniform, nor unidirectional.

The videos of the wounded area being filled in that are accessible on the Cell Migration Gateway interactive website are particularly illuminating (see videos at www.cellmigration.org/resource/discovery/brugge/simpson2008_rnai_time.cgi?uid=376). Wild type cells after wounding remained highly organized and tightly associated in a monolayer even when cells within the monolayer underwent mitosis. The vacant, wounded area was re-covered by the remaining cell monolayer moving slowly into the available space left by the wound, in an organized and unidirectional manner, with the cells maintaining close associations with each other and clear ruffled leading edges abutted to the open space. In contrast, the Cx43 knockdown cell monolayers showed disrupted adhesion, migration and polarity as noted above. Even at time zero, as the time-lapse imaging begins, the size and shape of the GJA1 knockdown cells are clearly different in that they appeared larger, more spread-out and irregularly shaped. While at wounding the cells start out as an adherent organized monolayer, and mitotic cells are observed within the monolayer, the region of the monolayer close to the wound dissociates into single cells which migrate rapidly from the wound edge and change direction frequently. These migrating cells show defects in the formation and the retraction of leading edges and large tails, and display multiple, large, broad protrusions per cell, that are oriented in different directions. While there are many ruffling leading edges, the cells move off quickly and the direction the individual cell moves changes, as if they have lost defined and stable polarity. Cells that are changing direction leave parts of themselves at their original location, adhered to the surface, and the cells/cell membranes stretch between the tail of the cell and the rest of the cell body as it moves off rapidly. Cells that move erratically in different directions come into the field of view from other regions distant to the wound area that is being imaged by microscopy, as opposed to the uniform left to right movement, in military precision, of wild type cells. Therefore it is clear that Cx43 had significant influences on cell adhesion, polarity and directed migration. Immunoblotting data in the supplementary figures show that the levels of the adhesion proteins N- and P-cadherin's are decreased in the GJA1 knockdown cells, while E-cadherin levels are raised. The lower amounts of two of these adhesive proteins could explain the lack of integrity of the cell monolayer and the inability of the cells to remain in a contiguous sheet as seen for the wild type cells. These findings are consistent with the findings of others that showed that N-cadherin and Cx43 are associated in a protein complex [44], and

that both are required for effective GJ formation between fibroblasts, and important (as will be discussed below) for cardiac neural crest cell motility [45]. These results are summarized in Fig. 2.

Numerous studies in epidermal wound healing have reported similar effects to those noted above for breast epithelial cells, meaning the enhanced motility of the cells that results in more rapid filling in of the wounded gap by migrating cells. Of particular interest are the reports of increased in vivo skin wound healing mediated through Cx43 antisense, siRNA or inhibitory peptides [46–48].

### 4.2. Cardiac neural crest cell migration

The results of Simpson et al. [43] agree with and support the findings from Cecilia Lo's lab where the migration of cardiac neural crest cells (CNC) and fibroblasts from Cx43 knockout and heterozygous mice was examined [45,49,50], in particular, the effect of Cx43 knockdown on the polarity of cell protrusions. Comparisons of these papers reveal interesting common themes.

Normal development of the heart involves complex migration of cardiac neural crest (CNC) cells in a defined, prototypical manner and this is disrupted in a variety of Cx43 knockout mouse models. A Cx43 null mutation results in perinatal lethality due to a disruption in cardiac development which resulted in a blockage of the right ventricular outflow track [30,51] which was later resolved to be due to a CNC cell migration defect [52]. GJs may mediate the cell-to-cell transport of second messengers and other cell-signaling molecules involved in regulating cell locomotion and in this manner help coordinate the deployment of CNC cells to the developing heart [51,52]. In addition the finding that N-cadherin-based adherens junctions are important for modulating GJ communication, often being found in close proximity to Cx43 GJs, indicates their potential role in the regulation of migration of mouse CNC cells [45]. In Xu et al. [49], CNC cells were obtained from wild type, Cx43 heterozygous ($Cx43^{+/-}$), Cx43 knockout ($Cx43^{-/-}$) and Cx43 re-expressing transgenic mice using a CMV promoter to drive re-expression (CMV-Cx43). Neural tubes were explanted from embryos and the CNC cells cultured. The CNC cells were assayed for a) motility on different concentrations of fibronectin, b) their directionality of movement, c) the number, extent and direction of protrusions, d) the effect on β1-integrin, e) the location of focal adhesions and actin stress fibers, f) the effect of Semaphorin 3a (Sema3a), and g) the immunofluorescence patterns of proteins associated with focal adhesions and the cytoskeleton (e.g. vinculin, ezrin and drebrin). Compared with wild type CNC cells, $Cx43^{-/-}$ CNC cells moved with less directionality and more slowly as the concentration of fibronectin increased (1 μg/ml to 50 μg/ml). Images were captured every 10 min over a 20 hour interval to assess directionality of movement and process extension. Large differences in protrusive activity were observed. Wild type Cx43 +/+ CNC cells show unidirectional process/protrusion formation that progresses forward in the same direction. In contrast $Cx43^{-/-}$ CNC cells have multi-directional protrusions, many of them reported by the authors to extend and retract. These processes are not in the same direction and thus they impede forward movement. Restoring Cx43 expression in the CMV-Cx43 CNC cells resulted in cells that appeared to be like wild type cells, with more unidirectional protrusions. In addition the authors described that CNC cells in the absence of Cx43 were less 'round', meaning that the cells appeared 'spikey'. The lack of unidirectional processes that facilitate forward movement of the cells is reminiscent of the findings in Simpson et al. [43] discussed above, where the knockdown of the GJA1 gene results in poor directionality and lack of clear leading (forward) and lagging (rear) edges of cells as Cx43 levels decrease. It is important to note, however, that the cells in Simpson's study [43] still express Cx43 while those in Xu et al. [49] were complete Cx43 knockout cells.

The cytoskeleton in $Cx43^{-/-}$ CNC cells is disrupted in these studies. β1-integrin and vinculin, important components of focal adhesions, were visualized by immunofluorescence microscopy and the peripheral localization of both is seen in a focal adhesion type pattern in the wild type CNC cells as well as the restored CMV-Cx43 CNC cells. However in the $Cx43^{-/-}$ CNC cells, there is less peripheral β1-integrin and corresponding vinculin staining and the focal adhesions are disorganized or absent. Consistent with this, actin stress fibers are severely disrupted in $Cx43^{-/-}$ cells, with some lacking focal adhesions at the end of the fibers and the fibers themselves in a polygonal shape instead of being in normal parallel arrangement. Sema3a is supposed to antagonize integrin activation on fibronectin and it mediates growth cone collapse in neurons. Treatment with Sema3a causes wild type CNC cells to have a decrease in protrusions and overall increase in cellular roundness, while in contrast, the $Cx43^{-/-}$ cells show little change, since they are already more rounded. Quick scans for actin-associated proteins like drebrin, to assess the co-localization with Cx43, identified

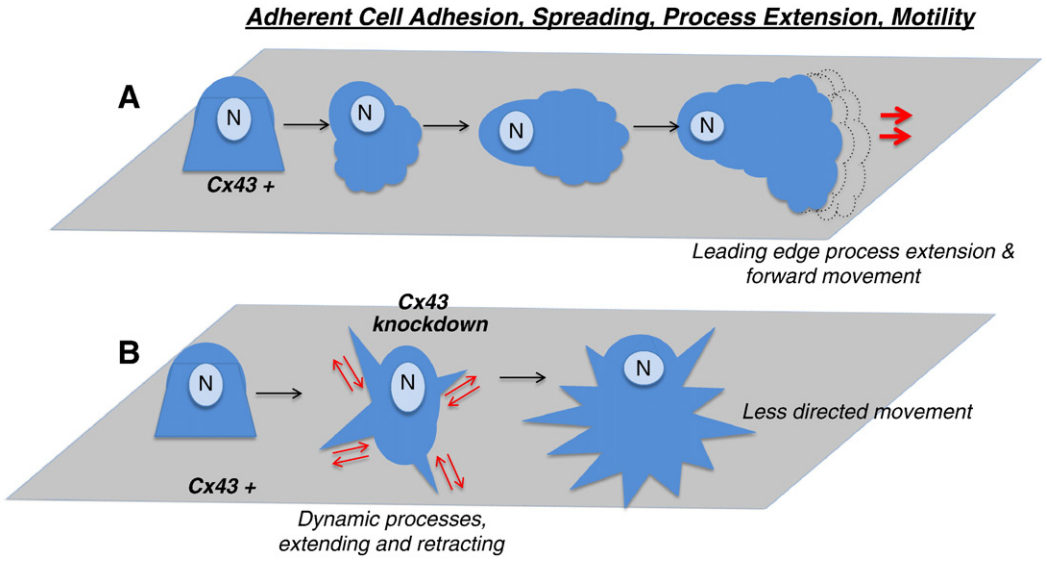


**Fig. 2.** Effect of Cx43 expression on adherent cells. Panel A. Cx43 expressing adherent cells (i.e. cardiac neural crest cells, breast endothelial cells, mouse embryo fibroblasts, glioma tumor cells) stick to and spread to surfaces and cell layers, extending processes, establishing polarity (leading and lagging edges of cells) that results in forward movement (red arrows). These cellular events involve rearrangements in cellular structure, for example the breakdown and reformation of the actin cytoskeleton. Gray circle indicates the nucleus (N). Panel B. Cx43 knockdown or knockout results in changes in cell morphology and the generation of dynamic processes that are often seen to extend and retract. The cells lack clear leading edges and have multi-directional processes that appear to impede directed forward movement.

some interesting localization patterns and pointed to candidates to examine in the future. These candidates would later be confirmed by the uncovering of a cytoskeletal platform of proteins as a result of a proteomic screen of Cx43 associated proteins in astrocytes [53], as well as in other reports of associated proteins identified by co-immunoprecipitation studies (reviewed in [54]).

The effect of Cx43 expression on cell polarity and directionality of movement and protrusions was further examined by comparing wild type mouse embryonic fibroblasts (MEF) with that from Cx43 $^{-/-}$ mice [50]. Cx43 deficiency causes cell polarity defects as characterized by a failure of the Golgi apparatus and the microtubule-organizing center (MTOC) to reorient with the direction of wound closure. MEFs from knockout mice do not close the wound as fast as wild type cells and the protrusions are more numerous, more pronounced and multidirectional, a reflection of a loss of polarity. Not only are there more protrusions, but these are less stable, retracting more frequently and the MTOC is not oriented in the direction of cell movement. Gain-of-function expression of Cx43 with a deletion of its tubulin-binding domain (the C-terminal domain; Cx43ΔT) in both wild type and Cx43 $^{-/-}$ MEFs or CNC cell explants recapitulates the cell migration defects seen in Cx43 knockout cells. Thus the cytoplasmic tail of Cx43 is important for the cytoskeletal rearrangements essential for cell movement and migration. Interestingly, expression of Cx43 with a point mutation causing GJ channel closure (mutant Cx43Y17S-GFP) has no effect on cell motility.

### *4.3. The role of gap junctions in migration in the developing nervous system*

GJs were first identified as electrical synapses in the nervous system [55] and thus the initial focus on the role they played was limited to that of an ion channel (reviewed in [56]). This seemed logical given the electrical nature of this tissue, and the characterization of many other ion channels important for the functioning of the nervous system. But it soon became apparent that many "non-electrical" tissues also had their cells connected via GJ channels and thus a more homeostatic role for this type of intercellular communication was proposed. With the identification of over 20 distinct GJ proteins or connexins with interesting tissue distributions and expression patterns that vary during cell differentiation and with differential localization in cellular membranes [57], it is clear that GJs are much more than channels.

In the central nervous system, up to 11 different Cx proteins have been shown to have not only specific spatial (cellular) distribution but also distinct temporal (developmental) profiles [58,59]. Given the changing expression of many of these Cxs associated with distinct processes during neural development, a number of approaches have been used to clarify the role these various Cxs might play. GJ channels and Cxs have been implicated in many aspects of neural development, including cell proliferation, migration, intra- and intercellular calcium signaling, electrical synapses and establishment of developmental gradients [60,61]. In this section of the review, we will focus specifically on the role of Cxs in neural cell migration.

The most abundant Cx expressed in the body, including the brain, is Cx43. Fortuitously this was the first Cx to be pursued in a targeted deletion strategy of GJ genes [30]. This revealed for the first time, a role for Cx43 in cellular migration, from two perspectives. First, the knockout of Cx43 resulted in a perinatal lethal phenotype due to a cardiac anomaly. Specifically, as discussed above, this was identified as a blockage of the right ventricular outflow track [30] which was later identified to be due to a CNC cell migration defect [51]. Second, the extensive levels of Cx43 in the developing brain suggested it could play a significant role in neural development [62–65]. In fact, it was shown that embryonic mice without Cx43 exhibited a delay in neuronal migration in the developing cerebral cortex, demonstrated by following the distance of migration of bromo-deoxyuridine pulse-labeled embryonic neuroblasts [5]. This latter phenotype in the developing brain was specifically followed up using two different genetic targeting strategies. The first involved knockdown of Cx expression in the rat embryonic brain [3], where it was shown that in-utero electroporation into the brain of shRNA plasmids targeting Cx43 or Cx26 resulted in a 50% knockdown of these proteins, and this was sufficient to cause a delay in neuronal migration in the developing cerebral cortex. The second approach involved a more direct genetic strategy, with conditional deletion of Cx43 using either nestin-cre [2] or GFAP-cre [6] transgenic mice crossed with Cx43-floxed mice. In nestin-cre:Cx43-floxed mice, where there was a 100% deletion of Cx43 in neural progenitor cells including radial glia, a significant delay in migration was observed [2]. These results were also confirmed in mice using Cx43 shRNA in-utero electroporation, as well as in the original parental line of Cx43 knockout mice [2]. In the study using GFAP-cre and Cx43-floxed mice, disruption of cerebral, hippocampal and cerebellar development consistent with migration defects was also reported, but this phenotype was strain-dependent [6]. These findings clearly establish that Cx43 is important for normal neuronal migration in the developing rodent cerebral cortex. These differences in neuronal cell orientation and migration are summarized in Fig. 3.

To further examine the mechanism by which Cx43 impacted neuronal migration, additional experiments were performed [3]. In the study by Elias et al. [3] various Cx43 and Cx26 expression-constructs were used to rescue the defect in migration due to the knockdown of these Cxs. First, it was demonstrated that the co-expression of plasmids encoding either Cx43 or Cx26 mRNAs with the respective shRNA constructs, mutated to be resistant to the shRNA-induced knockdown, was sufficient to rescue migration, demonstrating the importance and the specificity of these Cxs in the knockdown phenotype. It was found that the migration defect could also be rescued by expression of dominant-negative Cx43 and Cx26 channel mutants that, when assembled into complete connexon hexamers, resulted in inactive, closed channels [66]. These mutated Cxs were still able to traffic to the cell surface normally and make GJs. Expression of Cx43 with mutations in the cysteines in the extracellular domains [67] failed to rescue the migration defect, implying that the extracellular domains are important for the adhesive properties of GJs that are necessary for migration. However, these experiments were complicated by the fact that Cx43 cysteine mutants have deficiencies trafficking through the secretory pathway to the cell surface and thus have sparse plasma membrane localization [67]. Thus it is difficult to determine if the interpretation of these results is valid. Finally, to assess the role of the C-terminal domain in migration, Cx43 C-terminal truncation mutants (Cx43ΔCT) were expressed and found to be sufficient to rescue the migration defect, suggesting that any potential signaling or protein recruitment mediated by the cytoplasmic C-terminal domain does not have a significant role during radial migration. However, it should be noted that Cx43ΔCT mutants have been expressed in many different cell systems and there are no reports of trafficking deficiencies. Thus it is possible that the increased amount of Cx43 in the cell, including both mutant and wild type combined, resulted in the assembly of mixed mutant:wild type Cx43 heteromers that increased the overall levels of Cx43 on the cell surface. Overexpression of Cx43 at the plasma membrane can have significant effects and that could have been responsible for the rescue of the defect in neuronal migration. It is of interest to note that a subsequent report by the same authors claimed that the C-terminal domain of Cx43 is important for the tangential migration of developing interneurons, particularly when they switch direction from tangential to radial migration once they reach the area of the developing cortex [4]; see discussion below. ShRNA expressing neurons were also transplanted into the developing cortex of wild type recipient embryos and the same migration defect was shown, indicating that the neurons need to express these Cxs to migrate, and expression was not just limited to the radial glial cells. It was also shown that both Cx43 and Cx26 frequently co-localized with actin, positioning them in an ideal place to interact with the actin cytoskeleton, or to serve as a cytoskeletal protein recruitment platform, and impact migration. Overall, these experiments showed that Cx43 and Cx26 did not mediate neuronal migration through their channel

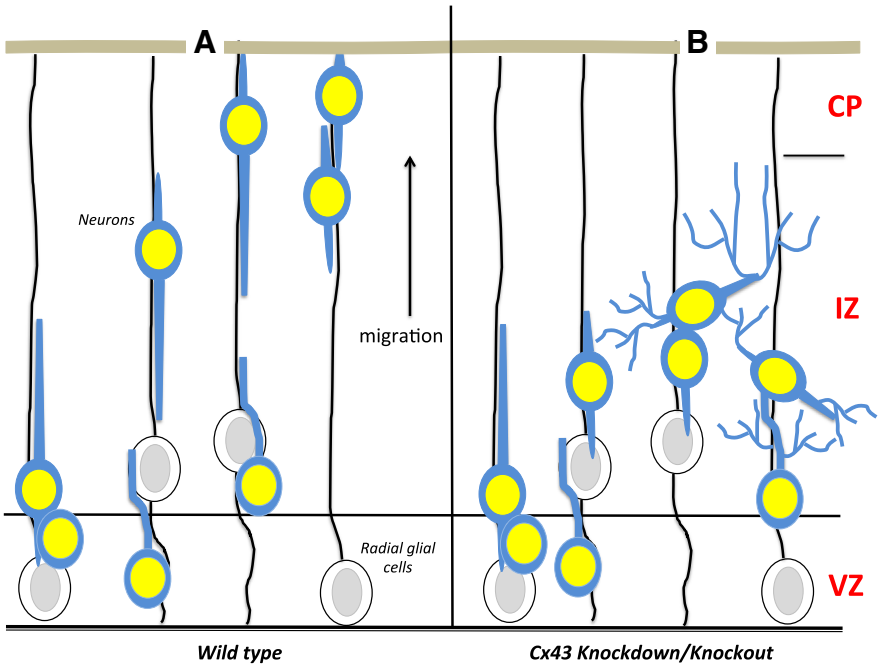


**Fig. 3.** Effect of Cx43 on the migration of neurons during cortical development. Panel A. In wild type mice, neurons (blue cells with yellow nuclei) migrate in a defined, uniform direction, along radial glial cells, from the ventricular zone (VZ), through the intermediate zone (IZ), reaching the cortical plate (CP). Panel B. In Cx43 knockdown or knockout mice, this process is disrupted. Instead, the well-defined, uniform migration of neurons to the cortical plate is inhibited, cellular organization is in disarray, and many never progress out of the intermediate zone by late gestation. In addition, the neurons are randomly oriented and they extend several, multi-directional processes.

properties. Instead it suggested that Cx43 could be a dynamic interacting component that influences the cytoskeleton to enable the stabilization of leading-edge processes along radial glial fibers, as well as the subsequent translocation of the nucleus via Cx26, the latter event an important part of neuronal migration. It was concluded by these authors that—"*gap junction adhesions play a part in stabilizing the leading process along a radial glial fibre. Indeed, Cx43-shRNA- and Cx26-shRNA-expressing neurons are unable to stabilize their processes and continue to extend multiple branches …. This phenotype is reminiscent of neural crest cells from Cx43KO mice that show increased protrusive activity but decreased directional migration.*" It is worth noting the common observation of both Elias et al. [4] and Cecilia Lo's group [49] in the change in the directionality of the protrusions/processes, depending on the levels of Cx43 expression. In fact, in the movies associated with Elias et al. [3] a clear difference is the complexity and the directionality of the growth and extension of processes was captured after Cx43 knockdown, with multi-directional processes that were dynamic dominating in the case of knockdown neurons, similar to the multi-directional protrusions seen in the CNC cells from Cx43 $^{-/-}$ mice in Xu et al. [49].

In the study by Cina et al. [2], most of the findings are consistent with those of Elias et al. [3], however this study used a genetic approach with Cx43 knockout mice and thus provides additional insight. Using a conditional deletion of Cx43 from neural progenitor cells, specifically by crossing nestin-cre mice with Cx43-floxed mice, it was demonstrated that Cx43 deletion resulted in disrupted radial migration of neurons in the developing mouse cerebral cortex (diagrammed in Fig. 3). The migration defect could be rescued by re-expressing wild type Cx43 in the cortex. However, in contrast to the report by Elias et al. [3], migration could not be rescued by expression of a C-terminal domain truncation (Cx43ΔCT) mutant. Since all the targeted neuronal cells in these animals only express the Cx43ΔCT mutated form of Cx43 and no wild type form, there is no possibility of increasing the total amount of wild type Cx43 on the plasma membrane (as possible in Elias et al. [3], noted above), since there is no wild type protein present to form mixed heteromeric connexons that could traffic to the cell surface. This genetic approach, using specific knockout mice to test the importance of the cytoplasmic tail of Cx43, leads to a more direct interpretation of the significance of this region in cell migration. In support of this finding, Cina et al. [65] also demonstrated that mice expressing only the Cx43ΔCT form of Cx43, and no wild type form in all tissues [68], also showed impaired neuronal migration.

There are several possible reasons for these differences related to the need for the Cx43 C-terminal domain for neuronal migration. The two studies discussed above used different species—rat versus mouse, and very different molecular approaches. Elias et al. [3] used shRNA rather than direct gene knockout, thereby only obtaining 50% partial knockdown. Therefore when they see migration rescue with Cx43ΔCT, there is still a substantial pool of wild type Cx43 present. They did not address how the persistent presence of wild type Cx43 would impact the rescue experiments, where various Cx43 and Cx26 mutant constructs are co-expressed.

One concern regarding all these studies is why a large number of neurons still make it to the right layers of the cortex. Cina et al. [2] showed that although the cortex looks rather "normal" by 2 weeks after birth, there is a persistent thinner layering consistent with a permanent effect of the migration defect and fewer cells being able to migrate to the correct location. Overall, the cortex of the adult mice appears normal, but there are a few neurological impairments reported, including increases in exploratory behavior, impairment of motor capabilities, and changes in brain acetylcholine levels [69], as well as acceleration of hippocampal spreading depression [70]. There may be a subpopulation of neurons that is independent of Cx43 for their migration; this has already been reported to be the case for interneurons whose tangential migration from the ganglionic eminence during development is not dependent on Cx43 until they make the switch to a radial trajectory when they reach the developing cortex [4]. Using a very similar shRNA approach to that of their earlier study [3] combined with similar types of rescue experiments, but done in cultured brain slices, it was shown that Cx43 and Cx26 are not needed for tangential neuronal migration. However Cx43 is needed for the switch from tangential to radial migration. Again, the evidence indicated that a functional GJ channel is not needed, but the presence of intact extracellular domains implied

that adhesion is necessary. Furthermore, in this case, Cx43ΔCT was unable to rescue the switch from tangential to radial orientation, suggesting a role for the C-terminal domain in this aspect of neuronal migration.

While most of the work has focused on Cx43, Cx26 has been shown to be involved in neuronal migration as well. Elias et al. [3] demonstrated that Cx26 knockdown impedes migration, but a different mechanism that regulates adhesion was suggested. While Cx43 is localized to leading edge areas of neuronal processes and areas where they contact radial glia, Cx26 is localized within the soma (cell body), is suggested to interact with the centrosome and to be involved in nuclear migration within the cell. This process is referred to as interkinetic nuclear migration (INM) and is closely associated with the organization of cell division. INM is a common feature of developing neuroepithelia, including the ventricular zone of the cortex, and consists of the periodic movement of the cell nucleus in phase with cell-cycle progression [71]. The neuroepithelial cells of the cortex undergo a finely orchestrated program of symmetrical (proliferative) or asymmetrical (neurogenic) division that is aligned with INM [72]. Therefore one might expect that disruption of INM disrupts overall cortical neurogenesis, perhaps influencing cell polarity, and thus also impacting neuronal migration. However the story is complicated by the fact that Cx43 has also been shown to play a role in this INM process [73] so thus both of these Cxs must be working in synergy in the INM process, a clear mechanism associated with the polarization of these cells including the polarization of the cellular cytoskeleton.

To help clarify the role of Cx26 in neuronal migration, a more recent study has focused on the role of focal adhesion kinase (FAK) in assembling Cx26 contact points in migrating neurons [74]. Since FAK has been shown to regulate the formation and disassembly of adhesions during the migration of multiple cell types [75,76], Valiente and colleagues assessed FAK in the distinct radial versus tangential neuronal migratory pathways in the developing cerebral cortex. They showed that FAK is required for radial migration of pyramidal cells where there are neuron–glial interactions, but not for tangential migration of inter-neurons which do not interact with radial glia. They investigated loss of FAK using a number of approaches (siRNA against FAK or conditional deletion, as well as specific deletion in interneurons) and showed that this disrupts radial migration but not tangential migration. In addition, when FAK was deleted, neurons in the radial migration pathway are more dispersed, with more processes per cell. Cx26 seems to preferentially mediate the interaction of the cell body and proximal leading process with radial glial cells, while Cx43 is enriched along the entire leading process. It has been suggested that FAK and Cx26 interact, since co-immunoprecipitation of Cx26 and FAK has previously been shown in prostate cells [77]. The authors suggest that Cx26 is a direct substrate for FAK, or that it has some role in protein complex formation to cause stability of cell contacts, possibly through interactions with ZO-1 which has been shown to be stabilized by FAK [78]. The authors suggest that FAK could act as scaffold protein, a function also suggested for Cx43 [31,79,80]. Valiente et al. [74] provide a helpful summary of our current understanding of the role of Cx26 as well as Cx43 in neuronal migration, stating: "*Considering the dynamic behavior of migrating neurons, it is expected that the assembly and disassembly of connexin-mediated adhesions would be a very dynamic process, but the mechanisms underlying this process have not been explored yet.*" Consistent with the findings that Cx43 deletion disrupts neuronal migration, Santiago et al. [81] reported that the absence of Cx43 results in premature neuronal maturation. In vivo, they observed an increase in β-III-tubulin immunoreactivity in the ventricular zone of developing Cx43 $^{-/-}$ mouse embryos, suggesting that the slowly migrating neuroblasts differentiate prematurely when Cx43 is absent; a similar effect was also seen in differentiating neurospheres. This effect could be reversed by restoration of Cx43 expression or expression of only the C-terminal domain. These authors go on to summarize how Cx43 may impact migration in a number of ways: a) "a ripple effect of GJA1 gene ablation on other genes" which affects migration, b) adhesion mediated by extracellular domains of Cx43, and c) C-terminal domain interactions with cytoskeletal scaffolding proteins and signaling cascades.

### *4.4. The importance of Cx43 in astrocytes and glioma cells*

While the importance of Cx43 in migration is best understood in the context of intact tissue systems or during developmental processes, Cx43 has been studied in a number of neural cell model cell systems. This has involved studies with normal glial cells, such as astrocytes, as well as with glioma cell lines. An early study on how Cx43 might mediate neural migration was carried out by Scemes et al. [82], using a migration assay applied to neurospheres produced from Cx43 wild type or Cx43 knockout mice. Once the neurospheres adhered to a substrate, the cells making up the sphere were given a chance to migrate away from the cell aggregate. Neurospheres composed of Cx43 knockout cells exhibited significantly reduced migration away from the aggregate compared to the migration from neurospheres composed of Cx43 wild type cells. These results are consistent with the in vivo reports of neuronal migration discussed above. Previous characterization of the Cx43 knockout cells revealed that they also had reduced expression of $P2Y_1$ receptors, important proteins involved in the exchange of ATP [83]. The re-expression of additional $P2Y_1$ receptors restored the ability of the Cx43 knockout cells to migrate. Consistent with this finding, the loss-of-function approach, by blocking $P2Y_1$ receptors in wild type cells, reduced migration to levels similar to Cx43 knockout cells. Coincident with these changes in $P2Y_1$ receptor expression and activation were changes in calcium signaling, an important second messenger involved in cell migration (reviewed in [84]). While in this system the $P2Y_1$ receptor appears to be a key player in migration, the role of Cx43 cannot be discounted since it has been shown to play a role in ATP release from cells, which could then act on these receptors in a paracrine or autocrine manner. Interestingly, a similar role in neuronal migration has been shown for $P2Y_1$ receptors in the developing mouse brain in vivo [85]. However $P2Y_1$ receptors have not been characterized in the Cx43 conditional knockout mouse brain [2] or the siRNA knockdown studies described above [3].

Cx43 is the major GJ protein in the brain, expressed primarily in astrocytes [64]. Therefore astrocyte culture systems have been extensively used to examine the role of Cx43 in glial cell function. With regard to the role of Cx43 in astrocytes, Olk et al. [53,54] have pursued a proteomic approach to assess how Cx43 might impact astrocyte cell functions, including shape changes and migration. They compared normal primary mouse astrocyte cultures with those treated with siRNA to down-regulate the expression of Cx43. Using gel electrophoresis in conjunction with MALDI-TOF mass spectrometry, they found 15 significantly regulated proteins (1.2 to 1.6-fold), of which 6 are known to belong to a group of cytoskeletal proteins involved in cortical platform formation. Using quantitative immunocytochemistry and Western blotting, they confirmed that astrocytes treated with Cx43 siRNA showed an increased expression of the cytoskeletal proteins actin, tubulin, tropomyosin, microtubule-associated protein RP/EB1, transgelin, and GFAP, and a decreased expression of cofilin-1 (Ser-3-phosphorylated cofilin). Cx43 silencing led to changes in cell morphology, migration, and adhesion. The observed shape change after Cx43 siRNA was consistent with the findings in Xu et al. [49] and Simpson et al. [43] discussed above, with lower expressing Cx43 cells having less directionality in terms of leading edges and multiple protrusions that go out in all directions and are more extended. Cx43 siRNA cells showed an increase in migration in transwell assays. With regard to adhesion, they found that Cx43 siRNA cells exhibited a 1.33 fold lower cell-to-cell adhesion capacity compared with control cells. This is consistent with the lack of adhesion among cells in other papers where Cx43 levels were lower [67], as well as pointing to the decrease in N-cadherin observed in previous studies [49].

Reviewing their work in the context of other literature dealing with Cxs, cytoskeleton and cell migration, Olk et al. [54,53] provide an overview of the main cytoskeletal proteins interacting with Cx43. Much of the proteomic work implies that many of the protein interactions impacting the cytoskeleton occur through domains on in C-terminal regions of Cx43 [45,46,80] as opposed to the intracellular loop or cytoplasmic amino terminal domain. The importance of the C-terminal domain in regulating cytoskeletal events has been supported by evidence showing that expression of only the C-terminal domain in L18 human glioma cells [86] has a dominant negative effect on cell function. The caution here however is that the C-terminal domain was not specifically targeted to the plasma membrane, nor to any intracellular membrane, and thus is not localized as the wild type Cx43 would be.

One tumor cell model used extensively in Cx43 studies is the rat C6 glioma tumor. These tumor cells normally express relatively low levels of Cx43, and thus were the first neural cells used to examine gain-of-function effects of Cx43 over-expression [87]. While the main focus of this initial paper was on the effects on cell proliferation, it was also clear that over-expression of Cx43 resulted in a morphological change in the cells, specifically the flattening and spreading out of normally spindle-shaped C6 cells and the enhancement of, or the increase in numbers of cell protrusions and processes [88,89]. Subsequent studies with C6 cells, that will be discussed next, clarified a number of aspects of how Cx43 expression levels could alter cell morphology, cell adhesion, cell migration and glioma tumor invasion [1,58,79,85].

Bates et al. [1] isolated C6 glioma subclones that expressed varying levels of Cx43. Higher levels of expression correlated with increased motility in wound healing and Transwell migration assays. Using shRNA to decrease Cx43 in the high expressing clones resulted in decreased motility using these two assays, while blocking the Cx43 channels had no effect on migration. It was also shown that transfection of the very low-expressing C6 glioma subclone with Cx43 to over-express the protein enhanced its ability to migrate in a wound healing assay, and to move through gelatin-coated transwell filters over a 24 hour period. Transwell migration was dependent on the C-terminal domain since expressing Cx43ΔCT in very low-expressing C6 glioma cells did not enhance cell movement in the migration assays like the effects achieved by over-expressing the wild type form of Cx43.

There have been several studies demonstrating an adhesive function for Cxs which support the close association of adhesion events with the Cxs, as noted above for Cx43 in neuronal migration [3]. Earlier work by Meyer et al. [90] demonstrated that GJ and adherens junction assembly can be inhibited by antibodies directed against Cx43 and A-CAM. Cx43 can associate with N-cadherin in the same protein complex in NIH3T3 cells, and blocking the effect of one will interfere with the proper membrane localization of the other one [44]. Lin et al. [67] examined the adhesive role of Cx43 in C6 glioma cells, specifically in the overall context of how this might impact glioma interaction with host astrocytes and invasion in the brain. In a short-term aggregation assay, Cx43 expression resulted in a 7-fold increase in the ability of C6 glioma cells to aggregate; this could be decreased in a dose-dependent manner using antibodies directed against the extracellular domain of Cx43. Therefore a clear role of extracellular domains for Cx43 in intercellular adhesion was demonstrated that was independent of channel function. These authors further examined the role of Cx43 in vivo, showing that implanted Cx43-expressing glioma cells established functional GJs with host astrocytes and dispersed through a substantially greater volume of brain parenchyma than mock- and mutant Cx43-transfected cells. This clearly supported a role for Cx43 in interactions between tumor cells and their surroundings, suggesting that both Cx proteins and their derived GJs were critical determinants of the invasiveness (and presumably the migration) of brain gliomas. Cx43 enabled glioma cells to establish GJs with host astrocytes and dramatically altered their pattern of invasion. C6 gliomas expressing Cx43 disseminated throughout the brain parenchyma, while control C6 cells migrated principally along the adluminal surfaces of the capillaries and blood vessels, presumably by making contact with these cells. The adhesive actions of Cx proteins did not require the formation of functional channels and thereby were distinct from their role in the assembly and maintenance of GJ intercellular communication. However, there is a need for functional GJs between glioma cells and the host astrocytes since a channel dead mutant, which still forms adhesive contacts, did not increase invasion. The authors suggested that an intercellular exchange of signals through GJs may be required for enhancing invasion.

It is clear that the strong non-covalent links established between docked connexons by the interactions of their extracellular domains are able to afford adhesive properties and thus also influence cell adhesion and migration. This adhesive function of extracellular domains could complement the functions of other domains of the Cx protein, including intracellular domains which interact with cytoskeletal and scaffolding proteins, as well as cytoplasmic domains that may influence motility-associated signaling pathways, as well as channel functions per se. Therefore when considering processes of tumor cell migration, invasion and metastasis, Cxs must be added to the factors that may play significant roles.

### *4.5. Immune system development and lymphocyte responses mediated by Cx43*

Cx43 is expressed in B-lymphocytes [91], but its function at the time of this original publication was not clearly understood. Cx43 $^{-/-}$ mice die perinatally from hypoxia due to malformation of the heart [92], making assessment of the adult immune system difficult. However, heterozygote Cx43 +/− mice did exhibit defects in T and B lymphocyte development [93,94]. It was not clear however if the defect was intrinsic to the B cells or due to defects in other cell types with which they interact. Lymphocytes and stromal cells do interact and are capable of establishing GJs but this was only true for a small portion of the cells examined in the study and there is still no evidence that GJ intercellular communication is an absolute requirement for establishment of the immune response [8,91–94]. However GJs are involved in the functioning of immune cells (see review [12]) and reported to contribute to the environment that hematopoietic stem cells require to find their specialized niches [95].

Given the role of Cx43 in cell adhesion, polarity and migration in fibroblasts, epithelial cells, CNC cells, C6 glioma and other neural cells, the expression patterns and the importance of Cx43 in lymphocytes have been examined [96,97]. It is important to note that lymphocytes are not monolayers of cells with adhesive junctions between cells, but instead exist as individual cells that can interact with other cells during development and the immune response. B and T lymphocytes move through the blood, lymph and tissues. Lymphocytes migrate across cell layers during development in the bone marrow, lymph nodes and spleen, they adhere to blood vessel walls and they extravasate through endothelial cell layers that line the vessel walls during development and during the immune response. Transendothelial migration is necessary to gain access to the various sites of inflammation and infection. In these studies by Machtaler et al. [96], two 'loss-of-function' approaches were used, one using shRNA-mediated knock-down of Cx43 in lymphocyte cell lines (WEHI 231 and A20), and the second using primary splenic B cells from heterozygous Cx43 $^{+/-}$ mice. Examination of knock-down lymphocyte cell lines and splenic B lymphocytes from Cx43 $^{+/-}$ mice showed that Cx43 was necessary for B cell antigen receptor (BCR)-induced membrane spreading on immobilized anti-BCR coated surfaces (which stimulate BCR signaling). The spreading of cell membranes in response to BCR signaling is thought to be a mechanism by which B cells can gather more antigens when interacting with antigen-presenting cells and the ultimate formation of the immune synapse [98–100]. The spreading response requires the breakdown of the actin cytoskeleton followed by its reformation as the cell membrane spreads outward. Similarly the retraction of the membrane that

normally occurs during this event also requires cytoskeletal alterations [101–104]. This cytoskeletal rearrangement by the B-lymphocyte is similar in principle to the formation of leading edges in adherent cells or the extension (and retractions) of large protrusions [101].

Cx43 expression was also required for lymphocyte adhesion to Bend3 brain endothelial cell monolayers, as well as motility, chemokine-mediated migration and extravasation through both Bend3 and SVEC4-10 endothelial cell monolayers [96,97]. Reduced expression of Cx43 correlated with impaired BCR-, chemokine (CXCL12)- and integrin (LFA-1)-activation of the Rap1 GTPase, a regulator of adhesion and migration in B-lymphocytes and other cell types [103,105,106]. To determine the role of Cx43 in these processes, wild type or mutated Cx43 was expressed in the myeloma (plasmacytoma) cell line J558μm3 that does not express endogenous Cx43. This exogenous expression was sufficient to enhance the normally low BCR-mediated Rap1 activation and lack of spreading responses by these cells, as well as the increased adhesion to endothelial cells. These effects were diminished when the cytoplasmic tail of Cx43 was truncated (using the same Cx43ΔCT mutant construct as in Bates et al. [1]), suggesting that this region of the protein was important [96], as previously highlighted by Bates et al. [1] and Francis et al. [50], discussed above. The expression of the C-terminal domain truncated Cx43 does not completely ablate the ability of the cell to extend processes. Process extension can occur but these are dynamic and lack stability, by repeatedly extending and retracting, the results of Machtaler et al. [96] are similar to the observations of Xu et al. [49] and Simpson et al. [43]. These results are summarized in Fig. 4 (please compare with Fig. 2). These data show that Cx43 plays an important and previously unreported role in regulating cytoskeletal organization and cytoskeleton-dependent processes in B-lymphocytes.

In lymphocytes a hint of the underlying molecular mechanism involved in the regulation of Cx43-dependent cytoskeletal rearrangements lies in studies with the actin adaptor protein HS1, the lymphocyte cortactin homolog. This is significant because cortactin is reported to be associated with Cx43 and proposed to be an important cytosolic effector mediating Cx43 effects [41]. HS1 deficient T lymphocytes fail to accumulate F-actin at the immune synapse and after T cell receptor signaling, which initiates signals similar to that of the BCR, the knockdown cells form actin-rich structures that are disordered, asymmetric, and unstable [107]. The disordered, multi-directional process formation of these T lymphocytes is reminiscent of the phenotype of process extension in the Cx43 knockdown cardiac neural crest cells, breast endothelial cells, fibroblasts and glioma cells discussed above [43,49]. In addition in natural killer cells, a type of T lymphocyte, phosphorylation of the tyrosine residue at position 397 (Tyr397) of HS1 was required for adhesion to the integrin ligand ICAM-1 and for cytolysis, whereas phosphorylation of Tyr378 was required for chemotaxis. Phosphorylation of Tyr397 was also required for integrin signaling and recruitment of integrins, adaptors and actin to the lytic synapse. Thus, HS1 is essential for signaling and actin assembly in natural killer cells, and the functions of the two phosphorylated tyrosine residues are distinct and separable [108]. Related to our discussion of process extension, in this study the morphology of the cells was also distinct depending on whether they were plated on I-CAM (integrin ligand of one type) or fibronectin. What is striking is the radial/lamellar spreading on ICAM versus the multi-directional processes that extend on fibronectin [108]. Similarly the multi-directional processes are reminiscent of the morphology of cells after Cx43 knockdown in various cell types [43,49] which points to possible targets for future examination to help explain the

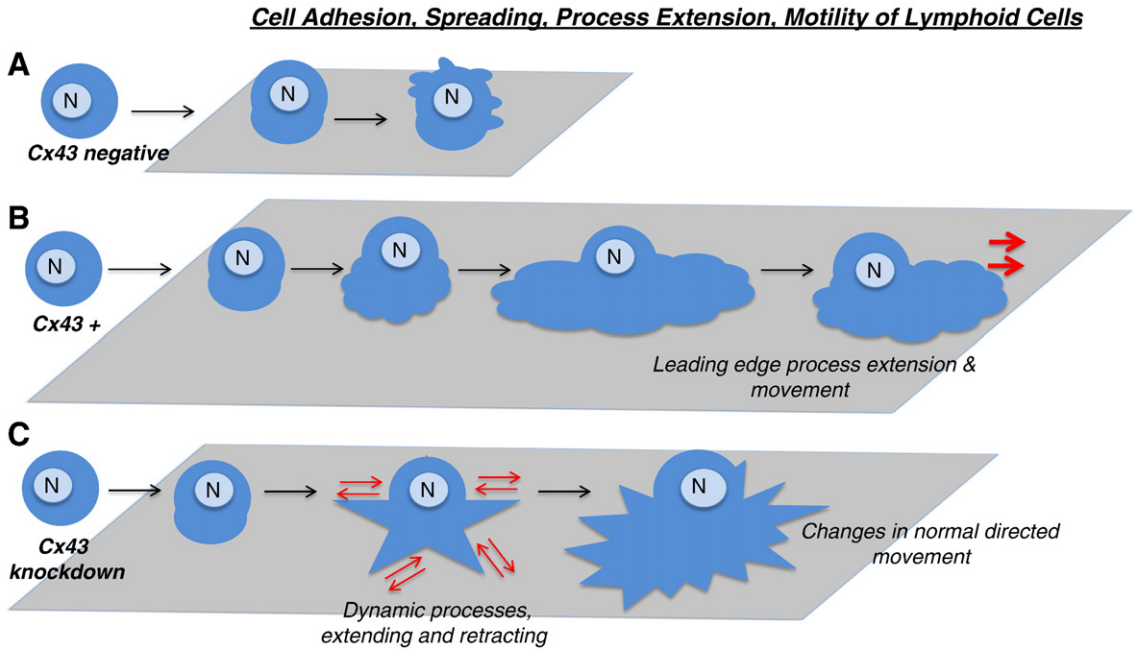


**Fig. 4.** Role of Cx43 on B-lymphocyte spreading and migration. Unless signals are received by cells, B-lymphocytes, especially antibody secreting plasmacytoma cells, are non-adherent and do not stick to tissue culture plates and coverslips. Panel A. Cx43 negative, B cell antigen receptor (BCR)-expressing, plasmacytoma cells will adhere and attempt to spread on anti-BCR coated surfaces, conditions which stimulate BCR signaling. However, without Cx43 expression, plasmacytoma cells are only able to produce small, stubby protrusions that do not necessarily contact that surface that the cells are resting on. Panel B. Cx43 positive, BCR-expressing lymphoid cells (either B-lymphoma cell lines or plasmacytoma cell lines transfected with Cx43) will adhere and spread their membranes radially in response to BCR or integrin signaling, changes that require reorganization of the actin cytoskeleton. In addition, B-lymphoma cells are able to rearrange their cytoskeleton, polarize and migrate in response chemokine receptor signaling. Panel C. If Cx43 expression in normally Cx43 positive lymphoid cells is knocked down by either shRNA or genetically reduced by using spleen cells from Cx43 heterozygous mice, the B cells will adhere and spread, however the spreading is either reduced, or the cell makes short dynamic processes that extend and retract. The overall result is an inhibition of forward movement as detected by a variety of motility assays. The same effects are seen with Cx43ΔCT as with knockdown of Cx43 in WEHI 231; there are dynamic protrusions that extend and retract. These results are similar to the effects of Cx43 knockdown in adherent cells shown in Fig. 2.

molecular mechanisms of the morphological differences in process extension.

## 5. Gap junctions and migration: involvement and implications in development and disease

In recent years several diseases have been attributed to GJ gene mutations (reviewed in [109–112]). While the disrupted cellular functions for some of these mutations are known (e.g. defects in channel gating, trafficking and turnover, membrane stability), there are many aspects of Cx function in the context of human diseases that remain to be explored, including the potential role of Cxs in abnormalities in cell movement and migration (reviewed here and in [113]).

Cell migration plays a major role in development, and thus it is not surprising, as discussed, that the first GJ gene (GJA1) deletion caused neonatal lethality [30]. In addition, the lack of Cx43 expression by the developing central nervous system results in impaired migration of neurons [2,3,5,114]. Human diseases associated with Cx43 mutations have been reported, including visceroatrial heterotaxia [114] and Oculodentodigital Dysplasia (ODDD) [115], reviewed in [116]. The heterotaxia findings have not been consistent in a larger cohort study [117]. It remains to be determined if the phenotype associated with the ODDD mutations involves cell migration defects. Interestingly in vitro wound healing studies show that fibroblasts from patients with ODDD have reduced migration [118]. In many other situations, it should be noted that the lack of developmental defects associated with Cx mutations and deletions could be due, at least in part, to the functional compensation by other Cx isoforms (reviewed in [119]). And thus, due to the multiple Cxs expressed in different cell types, the effect of Cx levels of expression on cell migration needs to be more carefully examined.

Much of the early literature about the clinical relevance of GJs focused on cancer (reviewed in [7,109,120]). While the initial focus was on their role in cell proliferation, one of the hallmarks of cancer progression involves cells breaking away from the tumor, moving through tissues, vasculature (transcellular migration) and the extracellular matrix and nucleating a new tumor at a different location [121]. Given the critical role of cytoskeletal rearrangements in cell migration, the reported interactions of Cx43 with many proteins involved in regulating the cytoskeleton [54,80,110] and the various effects of Cx43 knockdown or knockout on cell morphology, adhesion, polarity and movement that we have discussed here, Cx43 is placed as a key player in both developmental events and in the processes of tumor cell invasion and metastasis.

## 6. Summary

In this review we have discussed the common observations reported in various cell systems of the importance of Cx43 and other Cxs in processes that involve cytoskeletal changes. These processes include alterations in cell morphology, changes in cell adhesion, the determination of cell polarity, extension of protrusions and cell projections, cell movement, directed cell migration to a chemoattractant, and transcellular migration. The underlying common mechanisms that are involved in these processes are the initial breakdown of the existing actin cytoskeletal network matrix in order to change cell shape, extend a process or change the polarity in which the cell is oriented. This breakdown may be regulated in terms of location in the cell, thus generating polarized phenotypes as well as multiple regions where process extension occurs. After the initial breakdown of the actin cytoskeleton, the actin network is rebuilt. It may be linear or branched, but it must be differentially rearranged such that it can support a variety of different morphologies (i.e. thin processes, ones more flattened, more spread-out and adherent, multiple process extensions that dynamically extend and retract, or larger fan-shaped lamellapodia), a change in cell polarity (i.e. development of leading versus lagging edges of cells), and the complex sequence of events as cells migrate across surfaces, over other cell layers or through cell layers and tissues, sometimes changing direction during this process. Movement requires repeated breakdown and reformation of the cytoskeletal network in different locations in the cells, in a coordinated, sequential series of steps. These actin changes are mediated by a host of actin modifying enzymes and associated proteins that include cofilin, slingshot, LIM kinase, GTPases, WASP, ARP 2/3 complexes, among many others (reviewed in [122–126]).

What is observed regarding Cx43 expressing cells is that Cx43 knockdown or knockout has a profound effect on the ability to extend organized cell protrusions/projections/processes and to orient them in a direction that supports forward cell movement. The overall effect of the loss of function of Cx43 with regard to migration is reflected first in the lack of cell polarity, in the absence of organized movement and second, the inability to effectively migrate toward their target, despite the presence of chemoattractants. Consistent with this, gain of function studies have shown that cells that do not normally express Cx43, for example plasmacytoma cells, are less able to rearrange their actin cytoskeleton in an efficient and organized manner, compared with lymphoma cell lines that do express Cx43. Moreover, if Cx43 is expressed in Cx43-negative plasmacytoma cells, they can now respond to signals and rearrange their cytoskeleton, undergoing changes that result in adhesion and membrane spreading radially on solid surfaces [96,97]. What emerges from these and other studies is the idea that Cx proteins could also serve as a dynamic protein scaffold or “nexus” [127] for the association of proteins [53,54] that are involved in these types of cytoskeletal changes.

How could Cx43 act as a protein dynamic scaffold? It is clear from many studies that signaling receptors like the BCR and proteins like Cx43 inhabit unique domains in the plasma membrane. For example the BCR can be enriched in lipid raft domains [128,129] and the Cx proteins aggregate in regions to form plaques that contain many Cx hexamers (or connexons) that turn over rapidly ([130] also reviewed by Laird [7]). In some instances, superimposed on this membrane compartmentalization is the effect of the underlying actin cytoskeleton acting as a physical barrier, for example as a series of “picket fences”, that keep various proteins corralled in their appropriate membrane microdomains [102,131,132]. One possible way that Cx43 could contribute to the overall process of cytoskeletal rearrangement is to serve as a scaffold that binds to proteins that can sever actin or help rebuild it or form new branches and larger networks. There are several ways to think about this. First, Cx43 proteins or hexameric hemichannels contain motifs in their C-terminal domains that are modified in response to cell signaling [29,133,134] and have been correlated with the direct binding, or the association in a complex with, cytoskeletal interacting proteins (reviewed by [53,54]). In this way their C-terminal domains could contribute to the cohort of other scaffolds that exist in cells and that recruit cytoskeletal effectors. This could augment cytoskeletal rearrangements and/or regulate them. An alternate way to consider this is if membrane compartmentalization in microdomains normally restricts Cx43 proteins in regions devoid of potential modifying enzymes, then if Cx43 proteins are released after the first actin network breakdown Cx43 proteins are now allowed to change location and interact with modifying proteins, cellular responses could be initiated. This is similar to what is proposed for the BCR by [102]. If the Cx proteins are now able to freely move into different membrane microdomains and come in close proximity to other Cx43s where they associate with each other, or come in contact with enzymes (i.e. kinases) that modify the motifs on the Cx43 C-terminal domains, these modified Cxs could then act as more efficient scaffold proteins and bind proteins that can interact and modify the cytoskeleton. This would also enhance the process of cytoskeletal rearrangements. An additional interesting idea is the possibility that the intrinsic rapid turnover rate of Cx proteins in GJ plaques could contribute to the turnover of the actin network. In particular with Cx43, as these GJs are internalized and turned over, they are taken-up into the connexosome, removed from being in close proximity to the plasma

membrane and thus may no longer function effectively as a cytoskeletal protein scaffold. Finally, it is also intriguing to propose that there are two populations of Cx43 proteins at the cell surface; those that are involved in classical GJ intercellular communication plaques, passing small molecules in and out of cells, as well as a separate population whose C-terminal domains serve as dynamic scaffolds for the various associated proteins that regulate the cytoskeleton. This second population may be localized away from GJ plaques, have a longer life cycle in the cell and serve as effective dynamic scaffolds. In support of this concept, there is evidence for specialized domains of Cx43 both in the context of "formation plaques" [135,136] and the "perinexus" of cardiomyocyte GJs [38,39,137]. Thus in these various ways the actions of Cx43 proteins could be contributing not only to adhesion, spreading, cell shape determination and process extension, but also to the ability of cells to migrate during normal developmental and biological processes, as well as behaving abnormally in various disease processes.

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## References

[1] D.C. Bates, W.C. Sin, Q. Aftab, C.C. Naus, Connexin43 enhances glioma invasion by a mechanism involving the carboxy terminus, Glia 55 (2007) 1554–1564.
[2] C. Cina, K. Maass, M. Theis, K. Willecke, J.F. Bechberger, C.C. Naus, Involvement of the cytoplasmic C-terminal domain of connexin43 in neuronal migration, J. Neurosci. 29 (2009) 2009–2021.
[3] L.A. Elias, D.D. Wang, A.R. Kriegstein, Gap junction adhesion is necessary for radial migration in the neocortex, Nature 448 (2007) 901–907.
[4] L.A.B. Elias, M. Turmaine, J.G. Parnavelas, A.R. Kriegstein, Connexin43 mediates the tangential to radial migratory switch in ventrally derived cortical interneurons, J. Neurosci. 30 (2010) 7072–7077.
[5] S. Fushiki, J.L. Perez Velazquez, L. Zhang, J.F. Bechberger, P.L. Carlen, C.C. Naus, Changes in neuronal migration in neocortex of connexin43 null mutant mice, J. Neuropathol. Exp. Neurol. 62 (2003) 304–314.
[6] A.E. Wiencken-Barger, B. Djukic, K.B. Casper, K.D. McCarthy, A role for Connexin43 during neurodevelopment, Glia 55 (2007) 675–686.
[7] D.W. Laird, Life cycle of connexins in health and disease, Biochem. J. 394 (2006) 527–543.
[8] E. Oviedo-Orta, R.J. Errington, W.H. Evans, Gap junction intercellular communication during lymphocyte transendothelial migration, Cell Biol. Int. 26 (2002) 253–263.
[9] A. Bermudez-Fajardo, M. Yliharsila, W.H. Evans, A.C. Newby, E. Oviedo-Orta, CD4+ T lymphocyte subsets express connexin43 and establish gap junction channel communication with macrophages in vitro, J. Leukoc. Biol. 82 (2007) 608–612.
[10] R.J. Thompson, B.A. Macvicar, Connexin and pannexin hemichannels of neurons and astrocytes, Channels 2 (2008) 81–86.
[11] R. Iglesias, G. Dahl, F. Qiu, D.C. Spray, E. Scemes, Pannexin 1: the molecular substrate of astrocyte "hemichannels", J. Neurosci. 29 (2009) 7092–7097.
[12] J. Neijssen, B. Pang, J. Neefjes, Gap junction-mediated intercellular communication in the immune system, Prog. Biophys. Mol. Biol. 94 (2007) 207–218.
[13] A. Handel, A. Yates, S.S. Pilyugin, R. Antia, Gap junction-mediated antigen transport in immune responses, Trends Immunol. 28 (2007) 463–466.
[14] C.P.K. Lai, J.F. Bechberger, R.J. Thompson, B.A. MacVicar, R. Bruzzone, C.C. Naus, Tumor-suppressive effects of pannexin 1 in C6 glioma cells, Cancer Res. 67 (2007) 1545–1554.
[15] C.P.K. Lai, J.F. Bechberger, C.C. Naus, Pannexin 2 as a novel growth regulator in C6 glioma cells, Oncogene 28 (2009) 4402–4408.
[16] L.A. Musil, D.A. Goodenough, Multisubunit assembly of an integral plasma membrane channel protein, gap junction connexin43, occurs after exit from the ER, Cell 74 (1993) 1065–1077.
[17] P. Phelan, T.A. Starich, Innexins get into the gap, Bioessays 23 (2001) 388–396.
[18] E. Scemes, D.C. Spray, P. Meda, Connexins, pannexins, innexins: novel roles of "hemi-channels", Pflugers Arch. Eur. J. Physiol. 457 (2009) 1207–1226.
[19] G.E. Sosinsky, D. Boassa, R. Dermietzel, H.S. Duffy, D.W. Laird, B.A. MacVicar, C.C. Naus, S. Penuela, E. Scemes, D.C. Spray, R.J. Thompson, H.B. Zhao, G. Dahl, Pannexin channels are not gap junction hemichannels, Channels 5 (2011) 193–197.
[20] D.R. Cao, G. Lin, E.M. Westphale, E.C. Beyer, T.H. Steinberg, Mechanisms for the coordination of intercellular calcium signaling in insulin-secreting cells, J. Cell Sci. 110 (1997) 497–504.
[21] J.C. Saez, A.C. Nairn, A.J. Czernik, G.I. Fishman, D.C. Spray, E.L. Hertzberg, Phosphorylation of connexin43 and the regulation of neonatal rat cardiac myocyte gap junctions, J. Mol. Cell Cardiol. 29 (1997) 2131–2145.
[22] A. Baroja-Mazo, M. Barbera-Cremades, P. Pelegrin, The participation of plasma membrane hemichannels to purinergic signaling, Biochim. Biophys. Acta 1828 (2013) 79–93.
[23] N. Batra, R. Kar, J.X. Jiang, Gap junctions and hemichannels in signal transmission, function and development of bone, Biochim. Biophys. Acta 1818 (2012) 1909–1918.
[24] E. Scemes, Nature of plasmalemmal functional "hemichannels", Biochim. Biophys. Acta 1818 (2012) 1880–1883.
[25] R.A. Pearson, N. Dale, E. Llaudet, P. Mobbs, ATP released via gap junction hemichannels from the pigment epithelium regulates neural retinal progenitor proliferation, Neuron 46 (2005) 731–744.
[26] E. De Vuyst, N. Wang, E. Decrock, M. De Bock, M. Vinken, M. Van Moorhem, C. Lai, M. Culot, V. Rogiers, R. Cecchelli, C.C. Naus, W.H. Evans, L. Leybaert, Ca(2+) regulation of connexin 43 hemichannels in C6 glioma and glial cells, Cell Calcium 46 (2009) 176–187.
[27] D.A. Goodenough, D.L. Paul, Beyond the gap: functions of unpaired connexon channels, Nat. Rev. Mol. Cell Biol. 4 (2003) 285–294.
[28] M. Oyamada, Y. Oyamada, T. Takamatsu, Regulation of connexin expression, Biochim. Biophys. Acta 1719 (2005) 6–23.
[29] J.L. Solan, P.D. Lampe, Connexin43 phosphorylation—structural changes and biological effects, Biochem. J. 419 (2009) 261–271.
[30] A.G. Reaume, P.A. De Sousa, S. Kulkarni, B.L. Langille, D. Zhu, T.C. Davies, S.C. Juneja, G.M. Kidder, J. Rossant, Cardiac malformation in neonatal mice lacking connexin43, Science 267 (1995) 1831–1834.
[31] J.C. Herve, N. Bourmeyster, D. Sarrouilhe, H.S. Duffy, Gap junctional complexes: from partners to functions, Prog. Biophys. Mol. Biol. 94 (2007) 29–65.
[32] P.D. Lampe, A.F. Lau, Regulation of gap junctions by phosphorylation of connexins, Arch. Biochem. Biophys. 384 (2000) 205–215.
[33] P.D. Lampe, E.M. Tenbroek, J.M. Burt, W.E. Kurata, R.G. Johnson, A.F. Lau, Phosphorylation of connexin43 on serine368 by protein kinase C regulates gap junctional communication, J. Cell Biol. 149 (2000) 1503–1512.
[34] G.T. Cottrell, R. Lin, B.J. Warn-Cramer, A.F. Lau, J.M. Burt, Mechanism of v-Src- and mitogen-activated protein kinase-induced reduction of gap junction communication, Am. J. Physiol. Cell Physiol. 284 (2003) C511–C520.
[35] J.L. Solan, P.D. Lampe, Connexin43 in LA-25 cells with active v-src is phosphorylated on Y247, Y265, Y262, S279/282 and S368 via multiple signaling pathways, Cell Commun. Adhes. 15 (2008) 75–84.
[36] R. Lin, B.J. Warn-Cramer, W.E. Kurata, A.F. Lau, v-Src phosphorylation of connexin43 on Tyr247 and Tyr265 disrupts gap junctional communication, J. Cell Biol. 154 (2001) 815–827.
[37] B.N. Giepmans, W.H. Moolenaar, The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein, Curr. Biol. 8 (1998) 931–934.
[38] A.W. Hunter, R.J. Barker, C. Zhu, R.G. Gourdie, Zonula occludens-1 alters connexin43 gap junction size and organization by influencing channel accretion, Mol. Biol. Cell 16 (2005) 5686–5698.
[39] J.M. Rhett, J. Jourdan, R.G. Gourdie, Connexin43 connexon to gap junction transition is regulated by zonula occludens-1, Mol. Biol. Cell 22 (2011) 1516–1528.
[40] E. Butkevich, S. Hulsmann, D. Wenzel, T. Shirao, R. Duden, I. Majoul, Drebrin is a novel connexin-43 binding partner that links gap junctions to the submembrane cytoskeleton, Curr. Biol. 14 (2004) 650–658.
[41] R. Squecco, C. Sassoli, F. Nuti, M. Martinesi, F. Chellini, D. Nosi, S. Zecchi-Orlandini, F. Francini, L. Formigli, E. Meacci, Sphingosine 1-phosphate induces myoblast differentiation through Cx43 protein expression: a role for a gap junction-dependent and -independent function, Mol. Biol. Cell 17 (2006) 4896–4910.
[42] H. Asada, K. Uyemura, T. Shirao, Actin-binding protein, drebrin, accumulates in submembranous regions in parallel with neuronal differentiation, J. Neurosci. Res. 38 (1994) 149–159.
[43] K.J. Simpson, L.M. Selfors, J. Bui, A. Reynolds, D. Leake, A. Khvorova, J.S. Brugge, Identification of genes that regulate epithelial cell migration using an siRNA screening approach, Nat. Cell Biol. 10 (2008) 1027–1038.
[44] C.J. Wei, R. Francis, X. Xu, C.W. Lo, Connexin43 associated with an N-cadherin-containing multiprotein complex is required for gap junction formation in NIH3T3 cells, J. Biol. Chem. 280 (2005) 19925–19936.
[45] X. Xu, W.E. Li, G.Y. Huang, R. Meyer, T. Chen, Y. Luo, M.P. Thomas, G.L. Radice, C.W. Lo, Modulation of mouse neural crest cell motility by N-cadherin and connexin43 gap junctions, J. Cell Biol. 154 (2001) 217–230.
[46] D.L. Becker, C. Thrasivoulou, A.R. Phillips, Connexins in wound healing; perspectives in diabetic patients, Biochim. Biophys. Acta 1818 (2012) 2068–2075.
[47] C.S. Wright, M.A. van Steensel, M.B. Hodgins, P.E. Martin, Connexin mimetic peptides improve cell migration rates of human epidermal keratinocytes and dermal fibroblasts in vitro, Wound Repair Regen. 17 (2009) 240–249.
[48] G.S. Ghatnekar, M.P. O'Quinn, L.J. Jourdan, A.A. Gurjarpadhye, R.L. Draughn, R.G. Gourdie, Connexin43 carboxyl-terminal peptides reduce scar progenitor and promote regenerative healing following skin wounding, Regen. Med. 4 (2009) 205–223.
[49] X. Xu, R. Francis, C.J. Wei, K.L. Linask, C.W. Lo, Connexin 43-mediated modulation of polarized cell movement and the directional migration of cardiac neural crest cells, Development 133 (2006) 3629–3639.
[50] R. Francis, X. Xu, H. Park, C.J. Wei, S. Chang, B. Chatterjee, C. Lo, Connexin43 modulates cell polarity and directional cell migration by regulating microtubule dynamics, PLoS One 6 (2011) e26379.
[51] G.Y. Huang, A. Wessels, B.R. Smith, K.K. Linask, J.L. Ewart, C.W. Lo, Alteration in connexin43 gap junction gene dosage impairs conotruncal heart development, Dev. Biol. 198 (1998) 32–44.
[52] G.Y. Huang, E.S. Cooper, K. Waldo, M.L. Kirby, N.B. Gilula, C.W. Lo, Gap junction-mediated cell–cell communication modulates mouse neural crest migration, J. Cell Biol. 143 (1998) 1725–1734.
[53] S. Olk, A. Turchinovich, M. Grzendowski, K. Stuhler, H.E. Meyer, G. Zoidl, R. Dermietzel, Proteomic analysis of astroglial connexin43 silencing uncovers a

cytoskeletal platform involved in process formation and migration, Glia 58 (2010) 494–505.
[54] S. Olk, G. Zoidl, R. Dermietzel, Connexins, cell motility, and the cytoskeleton, Cell Motil. Cytoskeleton 66 (2009) 1000–1016.
[55] M.V. Bennett, E. Aljure, Y. Nakajima, G.D. Pappas, Electrotonic junctions between teleost spinal neurons: electrophysiology and ultrastructure, Science 141 (1963) 262–264.
[56] M.V. Bennett, R.S. Zukin, Electrical coupling and neuronal synchronization in the mammalian brain, Neuron 41 (2004) 495–511.
[57] G. Sohl, K. Willecke, Gap junctions and the connexin protein family, Cardiovasc. Res. 62 (2004) 228–232.
[58] C. Giaume, Astroglial wiring is adding complexity to neuroglial networking, Front. Neuroenergetics 2 (2010).
[59] G. Sohl, S. Maxeiner, K. Willecke, Expression and functions of neuronal gap junctions, Nat. Rev. Neurosci. 6 (2005) 191–200.
[60] R. Bruzzone, R. Dermietzel, Structure and function of gap junctions in the developing brain, Cell Tissue Res. 326 (2006) 239–248.
[61] B. Sutor, T. Hagerty, Involvement of gap junctions in the development of the neocortex, Biochim. Biophys. Acta 1719 (2005) 59–68.
[62] D.J. Belliveau, J.F. Bechberger, C.C.G. Naus, Isolation of neurons, astrocytes, and oligodendrocytes from brain to determine cell specificity of gap junction gene expression (Abstracts), Soc. Neurosci. 16 (Part 1) (1990) 349.
[63] T. Yamamoto, J. Vukelic, E.L. Hertzberg, J.I. Nagy, Differential anatomical and cellular patterns of connexin43 expression during postnatal development of rat brain, Brain Res. Dev. Brain Res. 66 (1992) 165–180.
[64] R. Dermietzel, O. Traub, T.K. Hwang, E. Beyer, M.V. Bennett, D.C. Spray, K. Willecke, Differential expression of three gap junction proteins in developing and mature brain tissues, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 10148–10152.
[65] C. Cina, J.F. Bechberger, M.A. Ozog, C.C. Naus, Expression of connexins in embryonic mouse neocortical development, J. Comp. Neurol. 504 (2007) 298–313.
[66] D.L. Beahm, A. Oshima, G.M. Gaietta, G.M. Hand, A.E. Smock, S.N. Zucker, M.M. Toloue, A. Chandrasekhar, B.J. Nicholson, G.E. Sosinsky, Mutation of a conserved threonine in the third transmembrane helix of alpha- and beta-connexins creates a dominant-negative closed gap junction channel, J. Biol. Chem. 281 (2006) 7994–8009.
[67] J.H. Lin, T. Takano, M.L. Cotrina, G. Arcuino, J. Kang, S. Liu, Q. Gao, L. Jiang, F. Li, H. Lichtenberg-Frate, S. Haubrich, K. Willecke, S.A. Goldman, M. Nedergaard, Connexin43 enhances the adhesivity and mediates the invasion of malignant glioma cells, J. Neurosci. 22 (2002) 4302–4311.
[68] K. Maass, A. Ghanem, J.S. Kim, M. Saathoff, S. Urschel, G. Kirfel, R. Grummer, M. Kretz, T. Lewalter, K. Tiemann, E. Winterhager, V. Herzog, K. Willecke, Defective epidermal barrier in neonatal mice lacking the C-terminal region of connexin43, Mol. Biol. Cell 15 (2004) 4597–4608.
[69] C. Frisch, M. Theis, M.A.D. Silva, E. Dere, G. Sohl, B. Teubner, K. Namestkova, K. Willecke, J.P. Huston, Mice with astrocyte-directed inactivation of connexin43 exhibit increased exploratory behaviour, impaired motor capacities, and changes in brain acetylcholine levels, Eur. J. Neurosci. 18 (2003) 2313–2318.
[70] M. Theis, R. Jauch, L. Zhuo, D. Speidel, A. Wallraff, B. Doring, C. Frisch, G. Sohl, B. Teubner, C. Euwens, J. Huston, C. Steinhauser, A. Messing, U. Heinemann, K. Willecke, Accelerated hippocampal spreading depression and enhanced locomotory activity in mice with astrocyte-directed inactivation of connexin43, J. Neurosci. 23 (2003) 766–776.
[71] E. Taverna, W.B. Huttner, Neural progenitor nuclei in motion, Neuron 67 (2010) 906–914.
[72] M. Gotz, W.B. Huttner, The cell biology of neurogenesis, Nat. Rev. Mol. Cell Biol. 6 (2005) 777–788.
[73] X.X. Liu, K. Hashimoto-Torii, M. Torii, C. Ding, P. Rakic, Gap junctions/hemichannels modulate interkinetic nuclear migration in the forebrain precursors, J. Neurosci. 30 (2010) 4197–4209.
[74] M. Valiente, G. Ciceri, B. Rico, O. Marin, Focal adhesion kinase modulates radial glia-dependent neuronal migration through connexin26, J. Neurosci. 31 (2011) 11678–11691.
[75] E. Avizienyte, M.C. Frame, Src and FAK signalling controls adhesion fate and the epithelial-to-mesenchymal transition, Curr. Opin. Cell Biol. 17 (2005) 542–547.
[76] S.K. Mitra, D.A. Hanson, D.D. Schlaepfer, Focal adhesion kinase: in command and control of cell motility, Nat. Rev. Mol. Cell Biol. 6 (2005) 56–68.
[77] A.W. Tate, T. Lung, A. Radhakrishnan, S.D. Lim, X. Lin, M. Edlund, Changes in gap junctional connexin isoforms during prostate cancer progression, Prostate 66 (2006) 19–31.
[78] E.R. Siu, E.W.P. Wong, D.D. Mruk, K.L. Sze, C.S. Porto, C.Y. Cheng, An occludin-focal adhesion kinase protein complex at the blood–testis barrier: a study using the cadmium model, Endocrinol. 150 (2009) 3336–3344.
[79] J.C. Herve, Gap junction channels: from protein genes to diseases, Prog. Biophys. Mol. Biol. 94 (2007) 1–4.
[80] J.C. Herve, M. Derangeon, D. Sarrouilhe, B.N. Giepmans, N. Bourmeyster, Gap junctional channels are parts of multiprotein complexes, Biochim. Biophys. Acta 1818 (2012) 1844–1865.
[81] M.F. Santiago, P. Alcami, K.M. Striedinger, D.C. Spray, E. Scemes, The carboxyl-terminal domain of connexin43 Is a negative modulator of neuronal differentiation, J. Biol. Chem. 285 (2010) 11836–11845.
[82] E. Scemes, N. Duval, P. Meda, Reduced expression of P2Y1 receptors in connexin43-null mice alters calcium signaling and migration of neural progenitor cells, J. Neurosci. 23 (2003) 11444–11452.
[83] E. Scemes, S.O. Suadicani, D.C. Spray, Intercellular communication in spinal cord astrocytes: fine tuning between gap junctions and P2 nucleotide receptors in calcium wave propagation, J. Neurosci. 20 (2000) 1435–1445.
[84] A.K. Howe, Cross-talk between calcium and protein kinase A in the regulation of cell migration, Curr. Opin. Cell Biol. 23 (2011) 554–561.
[85] X. Liu, K. Hashimoto-Torii, M. Torii, T.F. Haydar, P. Rakic, The role of ATP signaling in the migration of intermediate neuronal progenitors to the neocortical subventricular zone, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 11802–11807.
[86] S. Crespin, J. Bechberger, M. Mesnil, C.C. Naus, W.-C. Sin, The carboxyl-terminal tail of connexin43 gap junction protein is sufficient to mediate cytoskeleton changes in human glioma cells, J. Cell. Biochem. 110 (2010) 589–597.
[87] D. Zhu, G.M. Kidder, S. Caveney, C.C. Naus, Growth retardation in glioma cells cocultured with cells overexpressing a gap junction protein, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 10218–10221.
[88] C.C. Naus, D. Zhu, S.D.L. Todd, G.M. Kidder, Characteristics of C6 glioma-cells overexpressing a gap junction protein, Cell. Mol. Neurobiol. 12 (1992) 163–175.
[89] C.C. Naus, K. Elisevich, D. Zhu, D.J. Belliveau, R.F. Del Maestro, In vivo growth of C6 glioma cells transfected with connexin43 cDNA, Cancer Res. 52 (1992) 4208–4213.
[90] R.A. Meyer, D.W. Laird, J.P. Revel, R.G. Johnson, Inhibition of gap junction and adherens junction assembly by connexin and A-CAM antibodies, J. Cell Biol. 119 (1992) 179–189.
[91] E. Oviedo-Orta, T. Hoy, W.H. Evans, Intercellular communication in the immune system: differential expression of connexin40 and 43, and perturbation of gap junction channel functions in peripheral blood and tonsil human lymphocyte subpopulations, Immunol. 99 (2000) 578–590.
[92] Z.Q. Chen, D. Lefebvre, X.H. Bai, A. Reaume, J. Rossant, S.J. Lye, Identification of two regulatory elements within the promoter region of the mouse connexin43 gene, J. Biol. Chem. 270 (1995) 3863–3868.
[93] E. Montecino-Rodriguez, H. Leathers, K. Dorshkind, Expression of connexin43 (Cx43) is critical for normal hematopoiesis, Blood 96 (2000) 917–924.
[94] E. Montecino-Rodriguez, K. Dorshkind, Regulation of hematopoiesis by gap junction-mediated intercellular communication, J. Leukoc. Biol. 70 (2001) 341–347.
[95] A. Schajnovitz, T. Itkin, G. D'Uva, A. Kalinkovich, K. Golan, A. Ludin, D. Cohen, Z. Shulman, A. Avigdor, A. Nagler, O. Kollet, R. Seger, T. Lapidot, CXCL12 secretion by bone marrow stromal cells is dependent on cell contact and mediated by connexin43 and connexin45 gap junctions, Nat. Immunol. 12 (2011) 391–398.
[96] S. Machtaler, M. Dang-Lawson, K. Choi, C. Jang, C.C. Naus, L. Matsuuchi, The gap junction protein Cx43 regulates B-lymphocyte spreading and adhesion, J. Cell Sci. 124 (2011) 2611–2621.
[97] S. Machtaler, Role of the BCR and Connexin43 in B cell cytoskeletal rearrangements, (2012) PhD Thesis, University of British Columbia, January 2012.
[98] M.L. Dustin, A dynamic view of the immunological synapse, Semin. Immunol. 17 (2005) 400–410.
[99] S.J. Fleire, J.P. Goldman, Y.R. Carrasco, M. Weber, D. Bray, F.D. Batista, B cell ligand discrimination through a spreading and contraction response, Science 312 (2006) 738–741.
[100] F.D. Batista, N.E. Harwood, The who, how and where of antigen presentation to B cells, Nat. Rev. Immunol. 9 (2009) 15–27.
[101] M.L. Dustin, Hunter to gatherer and back: immunological synapses and kinapses as variations on the theme of amoeboid locomotion, Ann. Rev. Cell Dev. Biol. 24 (2008) 577–596.
[102] B. Treanor, D. Depoil, A. Gonzalez-Granja, P. Barral, M. Weber, O. Dushek, A. Burckbauer, F.D. Batista, The membrane skeleton controls diffusion dynamics and signaling through the B cell receptor, Immunity 32 (2010) 187–199.
[103] K.B. Lin, S.A. Freeman, S. Zabetian, H. Brugger, M. Weber, V. Lei, M. Dang-Lawson, K.W. Tse, R. Santamaria, F.D. Batista, M.R. Gold, The rap GTPases regulate B cell morphology, immune-synapse formation, and signaling by particulate B cell receptor ligands, Immunity 28 (2008) 75–87.
[104] S.A. Freeman, V. Lei, M. Dang-Lawson, K. Mizuno, C.D. Roskelley, M.R. Gold, Cofilin-mediated F-actin severing is regulated by the Rap GTPase and controls the cytoskeletal dynamics that drive lymphocyte spreading and BCR microcluster formation, J. Immunol. 187 (2011) 5887–5900.
[105] S.J. McLeod, M.R. Gold, Activation and function of the Rap1 GTPase in B lymphocytes, Int. Rev. Immunol. 20 (2001) 763–789.
[106] S.J. McLeod, A.H. Li, R.L. Lee, A.E. Burgess, M.R. Gold, The Rap GTPases regulate B cell migration toward the chemokine stromal cell-derived factor-1 (CXCL12): potential role for Rap2 in promoting B cell migration, J. Immunol. 169 (2002) 1365–1371.
[107] T.S. Gomez, S.D. McCarney, E. Carrizosa, C.M. Labno, E.O. Comiskey, J.C. Nolz, P. Zhu, B.D. Freedman, M.R. Clark, D.J. Rawlings, D.D. Billadeau, J.K. Burkhardt, HS1 functions as an essential actin-regulatory adaptor protein at the immune synapse, Immunity 24 (2006) 741–752.
[108] B. Butler, D.H. Kastendieck, J.A. Cooper, Differently phosphorylated forms of the cortactin homolog HS1 mediate distinct functions in natural killer cells, Nat. Immunol. 9 (2008) 887–896.
[109] C.C. Naus, D.W. Laird, Implications and challenges of connexin connections in cancer, Nat. Rev. Cancer 10 (2010) 435–441.
[110] D.W. Laird, The gap junction proteome and its relationship to disease, Trends Cell Biol. 20 (2009) 92–101.
[111] G. Zoidl, R. Dermietzel, Gap junctions in inherited human disease, Pflugers Arch. 460 (2010) 451–466.
[112] A. Pfenniger, A. Wohlwend, B.R. Kwak, Mutations in connexin genes and disease, Eur. J. Clin. Invest. 41 (2011) 103–116.
[113] P. Kameritsch, K. Pogoda, U. Pohl, Channel-independent influence of connexin 43 on cell migration, Biochim. Biophys. Acta 1818 (2012) 1993–2001.
[114] S.H. Britz-Cunningham, M.M. Shah, C.W. Zuppan, W.H. Fletcher, Mutations of the connexin43 gap-junction gene in patients with heart malformations and defects of laterality, New Eng. J. Med. 332 (1995) 1323–1329.
[115] A. Lai, D.N. Le, W.A. Paznekas, W.D. Gifford, E.W. Jabs, A.C. Charles, Oculodentodigital dysplasia connexin43 mutations result in non-functional

connexin hemichannels and gap junctions in C6 glioma cells, J. Cell Sci. 119 (2006) 532–541.
[116] W.A. Paznekas, S.A. Boyadjiev, R.E. Shapiro, O. Daniels, B. Wollnik, C.E. Keegan, J.W. Innis, M.B. Dinulos, C. Christian, M.C. Hannibal, E.W. Jabs, Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia, Am. J. Hum. Genet. 72 (2003) 408–418.
[117] G.Y. Huang, L.J. Xie, K.L. Linask, C. Zhang, X.Q. Zhao, Y. Yang, G.M. Zhou, Y.J. Wu, L. Marquez-Rosado, D.B. McElhinney, E. Goldmuntz, C. Liu, P.D. Lampe, B. Chatterjee, C.W. Lo, Evaluating the role of connexin43 in congenital heart disease: Screening for mutations in patients with outflow tract anomalies and the analysis of knock-in mouse models, J. Cardiovasc. Dis. Res. 2 (2011) 206–212.
[118] J.M. Churko, Q. Shao, X.Q. Gong, K.J. Swoboda, D.L. Bai, J. Sampson, D.W. Laird, Human dermal fibroblasts derived from oculodentodigital dysplasia patients suggest that patients may have wound-healing defects, Hum. Mutat. 32 (2011) 456–466.
[119] P. Bedner, C. Steinhauser, M. Theis, Functional redundancy and compensation among members of gap junction protein families, Biochim. Biophys. Acta 1818 (2012) 1971–1984.
[120] W.C. Sin, S. Crespin, M. Mesnil, Opposing roles of connexin43 in glioma progression, Biochim. Biophys. Acta 1818 (2012) 2058–2067.
[121] S. Valastyan, R.A. Weinberg, Tumor metastasis: molecular insights and evolving paradigms, Cell 147 (2011) 275–292.
[122] D.B. Billadeau, J.K. Burkhardt, Regulation of cytoskeletal dynamics at the immune synapse: new stars join the actin troupe, Traffic 7 (2006) 1451–1460.
[123] A.J. Ridley, Life at the leading edge, Cell 145 (2011) 1012–1022.
[124] C.A. Schoenenberger, H.G. Mannherz, B.M. Jockusch, Actin: from structural plasticity to functional diversity, Eur. J. Cell Biol. 90 (2011) 797–804.
[125] K. Rottner, T.E. Stradal, Actin dynamics and turnover in cell motility, Curr. Opin. Cell Biol. 23 (2011) 569–578.
[126] A. Michelot, D.G. Drubin, Building distinct actin filament networks in a common cytoplasm, Curr. Biol. 21 (2011) R560–R569.
[127] H.S. Duffy, M. Delmar, D.C. Spray, Formation of the gap junction nexus: binding partners for connexins, J. Physiol. Paris 96 (2002) 243–249.
[128] N. Gupta, A.L. Defranco, Lipid rafts and B cell signaling, Semin. Cell Dev. Biol. 18 (2007) 616–626.
[129] H.W. Sohn, P. Tolar, S.K. Pierce, Membrane heterogeneities in the formation of B cell receptor-Lyn kinase microclusters and the immune synapse, J. Cell Biol. 182 (2008) 367–379.
[130] A.L. Schubert, W. Schubert, D.C. Spray, M.P. Lisanti, Connexin family members target to lipid raft domains and interact with caveolin-1, Biochemistry 41 (2002) 5754–5764.
[131] B. Treanor, F.D. Batista, Organisation and dynamics of antigen receptors: implications for lymphocyte signalling, Curr. Opin. Immunol. 22 (2010) 299–307.
[132] B. Treanor, D. Depoil, A. Bruckbauer, F.D. Batista, Dynamic cortical actin remodeling by ERM proteins controls BCR microcluster organization and integrity, J. Exp. Med. 208 (2011) 1055–1068.
[133] J.L. Solan, P.D. Lampe, Connexin phosphorylation as a regulatory event linked to gap junction channel assembly, Biochim. Biophys. Acta 1711 (2005) 154–163.
[134] J.L. Solan, P.D. Lampe, Key connexin43 phosphorylation events regulate the gap junction life cycle, J. Membr. Biol. 217 (2007) 35–41.
[135] R. Johnson, M. Hammer, J. Sheridan, J.P. Revel, Gap junction formation between reaggregated Novikoff hepatoma cells, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 4536–4540.
[136] R.G. Johnson, J.K. Reynhout, E.M. TenBroek, B.J. Quade, T. Yasumura, K.G. Davidson, J.D. Sheridan, J.E. Rash, Gap junction assembly: roles for the formation plaque and regulation by the C-terminus of connexin43, Mol. Biol. Cell 23 (2012) 71–86.
[137] J.M. Rhett, R.G. Gourdie, The perinexus: A new feature of Cx43 gap junction organization, Heart Rhythm. 9 (2012) 619–623.
[138] M.L. Cotrina, J.H. Lin, M. Nedergaard, Adhesive properties of connexin hemichannels, Glia 56 (2008) 1791–1798.
[139] S.H. Graeber, D.F. Hulser, Connexin transfection induces invasive properties in HeLa cells, Exp. Cell Res. 243 (1998) 142–149.
[140] R. Oliveira, C. Christov, J.S. Guillamo, S. Debouard, S. Palfi, L. Venance, M. Tardy, M. Peschanski, Contribution of gap junctional communication between tumor cells and astroglia to the invasion of the brain parenchyma by human glioblastomas, BMC Cell Biol. 6 (2005) 7.
[141] W. Zhang, C. Nwagwu, D.M. Le, V.W. Yong, H. Song, W.T. Couldwell, Increased invasive capacity of connexin43-overexpressing malignant glioma cells, J. Neurosurg. 99 (2003) 1039–1046.
[142] Q. Shao, H. Wang, E. McLachlan, G.I. Veitch, D.W. Laird, Down-regulation of Cx43 by retroviral delivery of small interfering RNA promotes an aggressive breast cancer cell phenotype, Cancer Res. 65 (2005) 2705–2711.
[143] C. Qiu, P. Coutinho, S. Frank, S. Franke, L.Y. Law, P. Martin, C.R. Green, D.L. Becker, Targeting connexin43 expression accelerates the rate of wound repair, Curr. Biol. 13 (2003) 1697–1703.
[144] W.S. McDonough, A. Johansson, H. Joffee, A. Giese, M.E. Berens, Gap junction intercellular communication in gliomas is inversely related to cell motility, Int. J. Dev. Neurosci. 17 (1999) 601–611.
[145] J.M. Brandner, P. Houdek, B. Husing, C. Kaiser, I. Moll, Connexins26, 30, and 43: differences among spontaneous, chronic, and accelerated human wound healing, J. Invest. Dermatol. 122 (2004) 1310–1320.
[146] W.E. Li, K. Waldo, K.L. Linask, T. Chen, A. Wessels, M.S. Parmacek, M.L. Kirby, C.W. Lo, An essential role for connexin43 gap junctions in mouse coronary artery development, Development 129 (2002) 2031–2042.
[147] C.D. Moorby, A connexin43 mutant lacking the carboxyl cytoplasmic domain inhibits both growth and motility of mouse 3T3 fibroblasts, Mol. Carcinog. 28 (2000) 23–30.